International Journal of Fauna and Biological Studies
Available online at www.faunajournal.com

IJFBS
International Journal of Fauna And Biological Studies

ISSN 2347-2677
IJFBS 2019; 6(3): 55-59
Received: 23-03-2019
Accepted: 25-04-2019

Moura VC
Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

Moês APC
Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

Freitas AC
Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

Guillobel HCR
Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

Correspondence
Moura VC
Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

A photographic protocol for image sampling on the evaluation of color similarity between animal and substrate

Moura VC, Moês APC, Freitas AC and Guillobel HCR

Abstract
Camouflage has long intrigued scientists, who have been trying to quantify animal coloration. Recent advances in the field of computing allow the sampling and analysis of data from digital photographs. Thus, new quantitative studies on animal coloration have used these resources due to their practicality and accuracy. However, new methods and resources also bring new questions and challenges, in which improvement opportunities can be identified. In this study, we proposed a new sampling method and tested the influence of photographic parameters on a recent method for color similarity quantification between animal and substrate. Therefore, 135 digital photographs were obtained under controlled conditions and comparative tests were performed to test the proposed sampling method and differences caused by different photographic parameters. The results showed significant differences between the colors sampled with different methods and between photographs obtained under different photographic parameters.

Keywords: Scientific photography, colorimetry, camouflage, background matching, color similarity, crypsis.

Introduction
Animal coloration has always been of scientific interest, due to both its beauty and biological function. What is now known as cryptic coloration has been of interest to many scientists since XVIII century. Darwin's grandfather, Erasmus Darwin, wrote: "The colors of many animals seem adapted to their purposes of concealing themselves, either to avoid danger or to spring upon their prey" [1]. Since then, many studies have been developed in animal coloration, mainly trying to explain the mechanisms of function and the quantification of concealment coloration.

Recent work on camouflage and quantification of animal coloration have been using digital photography and image analysis software, due to its practicality and analytical power [2-4]. However, some studies also involve the use of expensive proprietary software, which end up making the use of this approach unaffordable.

The work of Samia (2015) developed an affordable way to measure color similarity between animal and substrate by extracting color frequencies from digital images through the ImageJ [5] and Color Inspector 3D [6] software and analyzing them with an R [7] coded mathematical function named Color Overlapping Index (COI). Based on this method we identified an improvement opportunity for color frequency sampling.

The developed method states that any selection tool available in the ImageJ software can be used to select the areas of the animal's body and substrate to be compared. However, fixed shape selection tools, like the rectangular or circular, do not allow the selection of irregular areas, such as animal bodies and their substrates, making it impossible to fully sample the colors present in animal and substrate. With the use of the polygonal selection tool, due to its ability to select areas of any size and shape, we developed a protocoled method which fully samples the color of both animal and substrate areas.

In addition to the sampling issue, we also considered another important factor, the photographic variables, such as the use of artificial light and the angle from which one photographs. Therefore, the present work performs tests of consistency in relation to the variables source of light, white balance and shooting angle and proposes a new protocoled method for photographing and color sampling.

Materials and methods

In order to test the influence of different variables of the color sample method and the study of photographic variables, we obtained images of anuran amphibians, of which 17 individuals were of the species *Rhinella ornata* Spix, 1824 and 10 individuals of the species *Thoropa miliaris* Spix, 1824. The specimens were conserved in alcohol and belonged to the collection of the Laboratório de Herpetologia, of the Department de Bio logia Animal of the Universidad Federal Rural do Rio de Janeiro.

All the photographs were taken under controlled conditions of light exposition, angle and white balance. The photographs were taken with a Canon EOS 7D Mark II camera and an external Canon Speedlite 430EX II flash, using as setting, ISO 400, f/8 aperture. Shutter speed, white balance and flash power were kept in auto mode.

Since white balance is a crucial step in the photographic pipeline, ensuring the proper rendition of photographs by eliminating colors created by different illuminations [8], we choose then to standardize the white balance in the post production, using as parameter a neutral color common to all the photographs.

A simulated substrate was crafted to be the closest to the natural environment of the species under study.

The sampling areas were selected using the polygonal selection tool, which allows delineating areas of any size and shape. The selection was made to follow the contour of the animal's body precisely, excluding areas of shade or any element that was not part of the animal's body (figure 1).

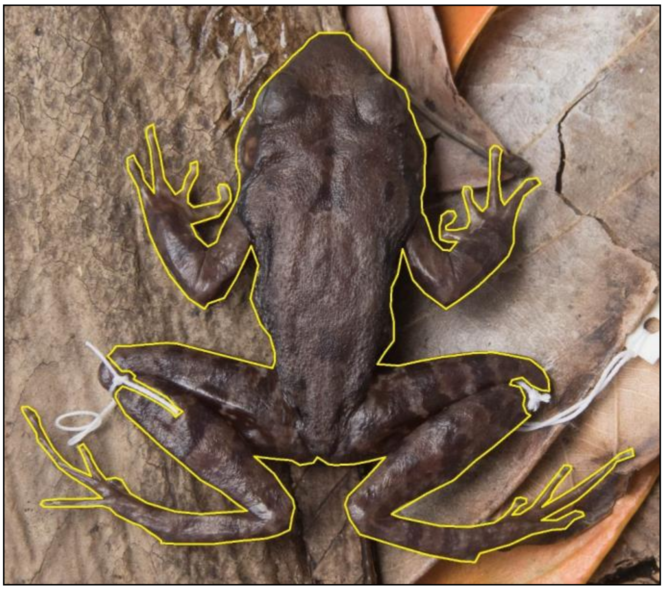

Fig 1: Selection (yellow line) of the animal's body area using the polygonal tool method.

The substrate area of each photograph was divided into four subareas, not necessarily equivalent in size. This method was chosen to facilitate the selection of irregular areas with obstacles or artifacts to be excluded. In addition, this approach makes it possible to detect the influence of different parts of the substrate allowing us to identify the parts of the substrate that are most similar to the animal. It was also defined that the contour lines of the substrate area were to be a few millimeters away from the contour of the animal's body area to avoiding overlapping the samplings (Figure 2).

Fig 2: Selection (yellow lines) of the substrate area using the Polygonal tool method.

To test the consistence between sampling methods, the photographs were also sampled with the rectangular tool, as described in Samia (2015).

Aiming to test each variable of the photograph, each individual was photographed in three different situations, at 90° angle under natural light, at 90° angle under artificial light and at 45° angle under artificial light. The white balance of all photographs was standardized in the post-production, using Photoshop Lightroom software (Adobe Systems Inc., USA).

The photographs taken at 90° angle, under artificial light were determined as the control group. Artificial light was chosen as control because it ensures that all photographs are taken with light of the same color.

Four groups were then sorted according to their specific parameters: photographs taken under natural light; photographs which white balance was not standardized; photographs taken at 45°angle; photographs sampled with a fixed shape selection tool, totaling 135 photographs.

The color information of all selected areas, from both the animal's body and the substrate, were then obtained through the Color Inspector 3D and exported in the form of data tables. The data were then inserted into the R platform and analyzed through COI function, which results vary between 0% (no color similarity) and 100% (full color similarity).

Student's t-test at a significance level of 5% was used to compare the test groups to the control group.

Results

The COI function analysis generated five results, four for polygonal sampling and one for rectangular sampling.

The mean of the four values generated by the polygonal sampling and the value generated by the rectangular sampling, were analyzed through student's t-test and plotted in dispersion graphs (figures 3 and 4).

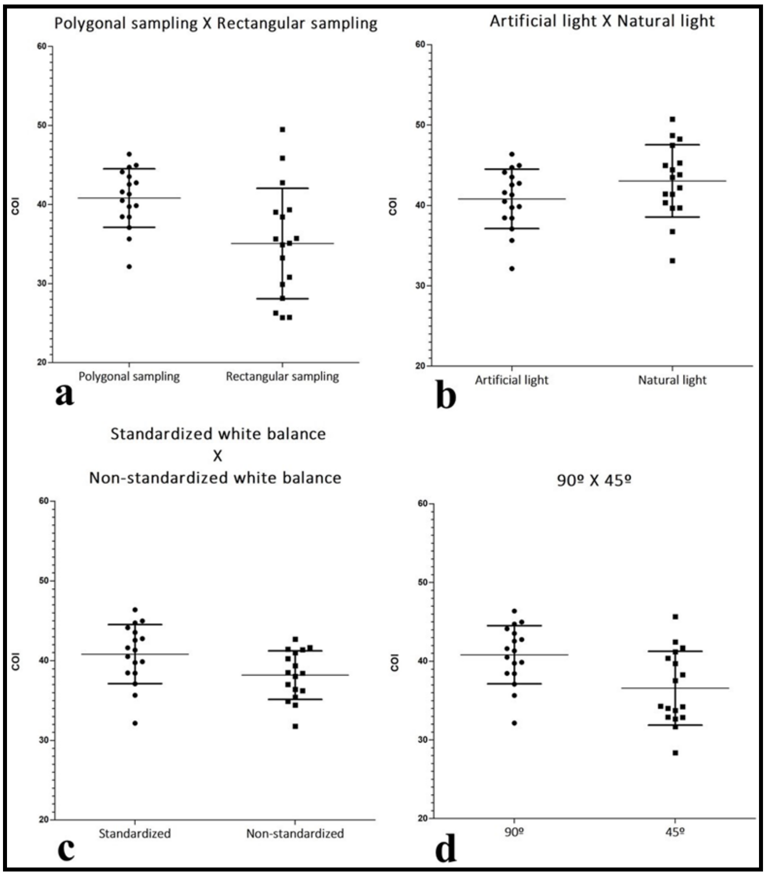

Fig 3: Graphs of the distribution of the COI function results for *Rhinella ornata* with mean values and standard deviation of one δ for the tests of (a) sampling; (b) light; (c) white balance; (d) shooting angle.

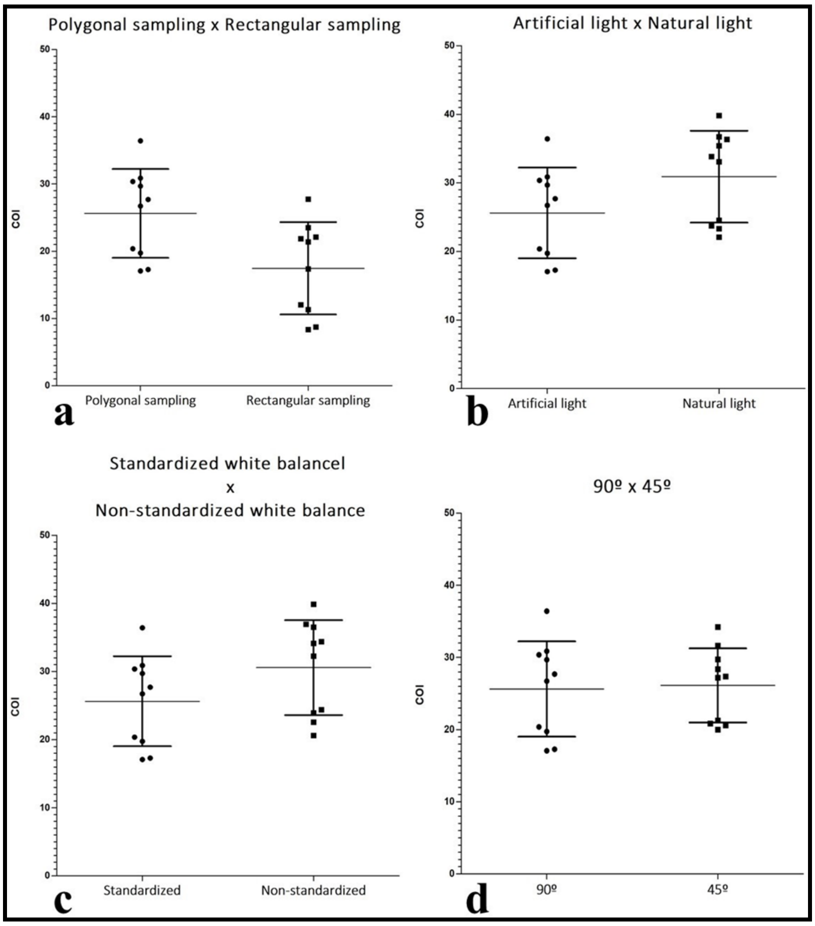

Fig 4: Graphs of the distribution of the COI function results for *Thoropa miliaris* with mean values and standard deviation of one δ for the tests of (a) sampling; (b) light; (c) white balance; (d) shooting angle

In the sampling test for the *Rhinella ornata* (figure 3), mean COI was 40.8% and 35.06% when sampled with the polygonal and rectangular method. For the *Thoropa miliaris* (figure 4), mean COI was 25.62% and 17.45% when sampled with the polygonal and rectangular method respectively. Results for both species differed statistically.

In the light test for the *Rhinella ornata,* mean COI was 40.8% and 43.04% when illuminated with artificial light and natural light respectively. For The *Thoropa miliaris,* mean COI was 25.62% and 30.90% when illuminated with artificial light and natural light respectively. Results of both species differed statistically.

In the white balance test for the species *Rhinella ornate*, mean COI was 40.8% and 38.16% with standardized and non-standardized white balance respectively. For the *Thoropa miliaris*, mean COI was 25.62% and 30.57% with standardized and non-standardized white balance respectively. Results of both species differed statistically.

In the angle test, for the species *Rhinella ornate,* mean COI was 40.8% and 36.55% when photographed at 90º and 45º angles respectively. For the species *Thoropa miliaris*, mean COI was 25.62% and 26.12% when photographed at 90º and 45º angles respectively. Only the results of the *Rhinella ornata* differed statistically.

Discussion

The results demonstrated that the color sampling method employed significantly altered the result of the Color Overlapping Index. This is probably due to the fact that the polygonal method sampled the entire photograph, regarding all animal and substrate colors, while fixed-shape methods, such as rectangular sampling, neglected part of the colors present in the animal and in the substrate. Since the COI function considers the frequency of the colors shared by animal and substrate, a full sampling of these areas should be considered.

The results showed that the colors shared by animal and substrate varied unevenly when under natural and artificial light. This makes photographs taken under different illuminations incomparable between each other. Light variations also occured in photographs taken under natural light at different times of the day, as the color of natural light changes according to the time and weather of the moment the photograph is taken. Therefore, the use of artificial light is the only way to ensure that all photographs are made under the same light condition, ensuring control over this variable, thus its use must be considered in order to guarantee the comparability of the photographs when using the COI function.

The results demonstrated that the standardization of white balance in photographic post-production affects the color of the animal and the substrate unevenly, making photographs with different white balance settings incomparable. Thus, the standardization of white balance should be considered to minimize the influence of the equipment in-built white balance algorithms when using the COI function.

The results demonstrated that the angle from which the photograph is taken can influence the color similarity between the animal and the substrate, depending on the color homogeneity and the body plan of the animal. Since the COI function weights the relative frequencies of each color shared by animal and substrate, an animal with larger flanks may have its results altered by the angle of the photograph. However, in an animal with flat body, because the flank area is small in relation to the rest of the body, the influence of the angle from which the photograph is taken is reduced. Therefore, the angle from which the animals are photographed should be determined considering their body plan and the study objective. For example, studies about predation of amphibians whose main predators are birds and snakes, with high and low view angles, respectively, should take these as the most indicated for photographing.

Data from all tests showed that the color sampling method, the source of light, the photographic equipment settings and the photographed angle have the potential to alter the color relation between the animal and the substrate perceived through the photograph. These factors suggest that for the applications of this method, which aim to measure coloration similarity through photography, one should consider the

Following protocol for photographic and color sampling:

1. Use the same ISO value for all photographs and the lowest ISO possible. This aims to guarantee the comparability of the photographs and the low ISO ensures low noise rates.
2. Set white balance for flash mode when working with flash or for the artificial light in use. For white balance standardization in post-production, the photographs should be taken with a standard reference, for example, a color checker. The use of the same white balance and its standardization in the post-production meant to lower its influence on colors of different photographs.
3. Use flash sync shutter speeds, between 1/125 and 1/250, if needed, shutter speeds below 1/125 must be done with stabilized equipment. The use of high shutter speeds and the stabilization of the equipment ensures steady clear images.
4. Prioritize small diaphragm apertures, like f/8 or smaller. Closer diaphragm apertures ensure the focus on both animal and substrate, avoiding unfocused parts on the photograph.
5. Shooting angles should be chosen based on the body plan of the object of study. This aims to sample the body portion which is seen more often in its natural habitat.
6. Photographs should be taken in raw format and then converted to a format capable of being analyzed. Taking photographs in raw format provides more color and texture information on the image files.
7. The areas of the photograph should be sampled using the polygonal method described in this work. The polygonal method as described samples all the color of the animal and substrate areas, providing a more accurate result.

Conclusion

The COI function method is an affordable and accurate way to measure color similarity. However, the color sampling method, the photographic equipment configuration, the light source and the photographed angle can significantly alter the results of the Color Overlapping Index. Thus we conclude that a pre stablished protocol, like the one proposed above, should be followed, making photographs comparable between each other and ensuring the most accurate results possible.

Acknowledgments

Thanks to the laboratório de herpetologia da Universidade Federal Rural do Rio de Janeiro for the logistical support and material provided for the accomplishment of this study. We are very grateful to Professor Dr. Helio Ricardo da Silva for providing the animals photographed in this study as well as valuable information about them. Special thanks to Professor Dr. Timothy Moulton for grammatically revising this paper.

References

1. Darwin, E. Zoonomia. Edn Johnson, London, 1794; 1:401.
2. Kang C, Stevens M, Moon J, Lee S, Jablonski PG. Camouflage through behavior in moths: the role of background matching and disruptive coloration. 2015; 26:45-54.
3. Samia DSM, Francini RB. An affordable method to measure animal-background contrast using digital images. International Journal of Fauna and Biological Studies. 2015; 2(1):8-16.
4. Troscianko J, Skelhorn J, Stevens M. Quantifying camouflage: how to predict detectability from appearance. BMC Evolutionary Biology. 2017; 17(1):7.
5. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to Image J 25 years of image analysis, Nature methods. 2012; 9(7):671-675. PMID 22930834.
6. BARTHEL KU. Color Inspector 3D v. 2.3, 2007 (http://rsb.info.nih.gov/ij/plugins/color-inspector.html).
7. Core Team R. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2016.
8. Paris S, Durand F, Avidan S, Hsu E, Mertens T. Light mixture estimation for spatially varying white balance. ACM Transactions on Graphics. 2008; 27:3(1).