University of Minnesota Morris Digital Well

4-14-2018

Can Diet Affect Coloration in Tiger Salamanders?

Katherine Novak
University of Minnesota - Morris, novak423@morris.umn.edu

Can Diet Affect Coloration in Tiger Salamanders?

University of Minnesota
MORRIS

Katherine Novak
Mentor: Heather Waye
University of Minnesota Morris

Introduction

Amphibians are the fastest known declining taxonomic group in the world with 48% of populations in decline (Stuart et. al. 2004). These declines in amphibian populations are partially due to the lack of basic life history data (Semlitsch 2003). For example, amphibians are known for their bright coloration and ability to rapidly change color. Coloration is key to amphibian survival in terms of defense (as camouflage or a warning sign to predators), thermoregulation (darker skin warms faster), and communication (primarily mating displays) (Rudh and Qvanstrom 2013). One aspect of amphibian coloration that is poorly understood is how much the environment influences amphibian pigmentation.

Amphibian coloration is dictated by three main types of chromatophores underneath the skin. Chromatophores are cells that contain pigments which reflect light. Iridophores contain pigments that reflect light that contribute to bluish coloration. Melanophores are the cells that control expression of melanin that can be expanded and contracted to darken or lighten the skin. Xanthophores are the cells responsible for the red and yellow coloration (Bagnara et. al. 1968) and contain pteridine organelles and dietary carotenoid pigments (Bagnara et. al. 1968 and Kraemer et. al. 2012). There are 600 different derivatives of carotenoids that have two major uses. They can be metabolized into Vitamin A and other antioxidants or used to physiologically change pigmentation. In particular, they are well known for being in orange and yellow vegetables like carrots and causing the bright pink coloration of the skin of some gulls. This coloration of gulls is due to their high-carotenoid diet of shrimp and algae (McGraw and Hardy 2006). Allocating carotenoids toward pigmentation rather than toward Vitamin A is a potential sign of health since that organism is able to use these nutrients for coloration instead of as antioxidants (Hill and Johnson 2012). This suggests that increasing access to carotenoids through diet can affect pigmentation which can reflect health in some organisms. We want to know whether carotenoids consumed through diet will affect pigmentation of amphibians as well.

Professor Heather Waye conducted research studying whether spot patterns in Eastern Tiger Salamanders (*Ambystoma tigrinum)* changed during maturation (Waye 2013). She made the observation that some of the salamanders' yellow coloration faded during their time in captivity. This may have been caused due to differences in abiotic factors (e.g. lighting, temperature) or diet. Captive salamanders have been fed a diet limited to domestic crickets, fish, and earthworms. Adult salamanders in the wild have been found to eat earthworms, crickets, grasshoppers, small mice, other amphibians (including their own larvae), mollusks, and other small insects (Reese 1969 and Moriarty and Hall 2014). Mollusks have been found to contain carotenoids (Vershinin 1996). I hypothesized that adding carotenoid-rich food to their diet will result in an increase in intensity of the yellow in their skin.

Materials and Methods

I took the 12 Tiger Salamanders (*Ambystoma tigrinum*) who have been individually housed at University of Minnesota Morris and ran a diet experiment. Six salamanders were fed with silverside fish as the control group and six were fed with Rod's Food Krill+ Blend as the experimental group. Group selection was based on initial weights and gender. Before beginning the experiment, to control for outside stimuli, I cleaned each terrarium, replaced each of the salamander's moss and light bulb, and covered the outside of each terrarium with black paper. Initial weights and photographs were taken. Every week for the past seven weeks each salamander has been fed twice a week with a total of approximately 2.5% of their body weight. The salamanders were re-weighed every week before feeding. The salamanders' dorsal and ventral areas were photographed each week under a standard lighting with a color standard for later data analysis (Figures 1 and 2). All photographs were taken with a Canon PowerShot SX40 HS.

Preliminary data analysis was done with an online image color summarizer on the color standard as well as on two random non-black spots on the dorsal side of the salamander. The hue value generated was compared relative to the hue value of the standard and then the initial photographs to photographs taken at Week 7 hue values were compared. The hue value was used as the factor of comparison because it can be used as a value for color.

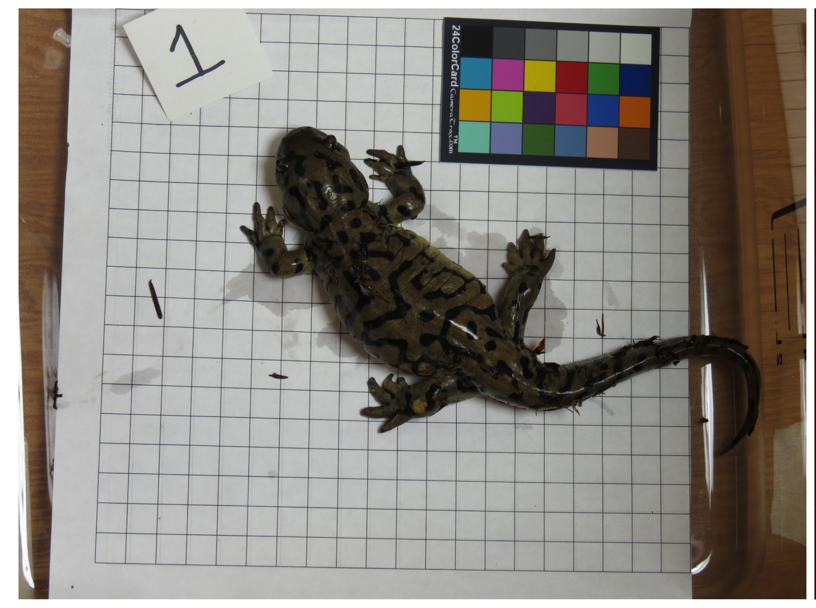


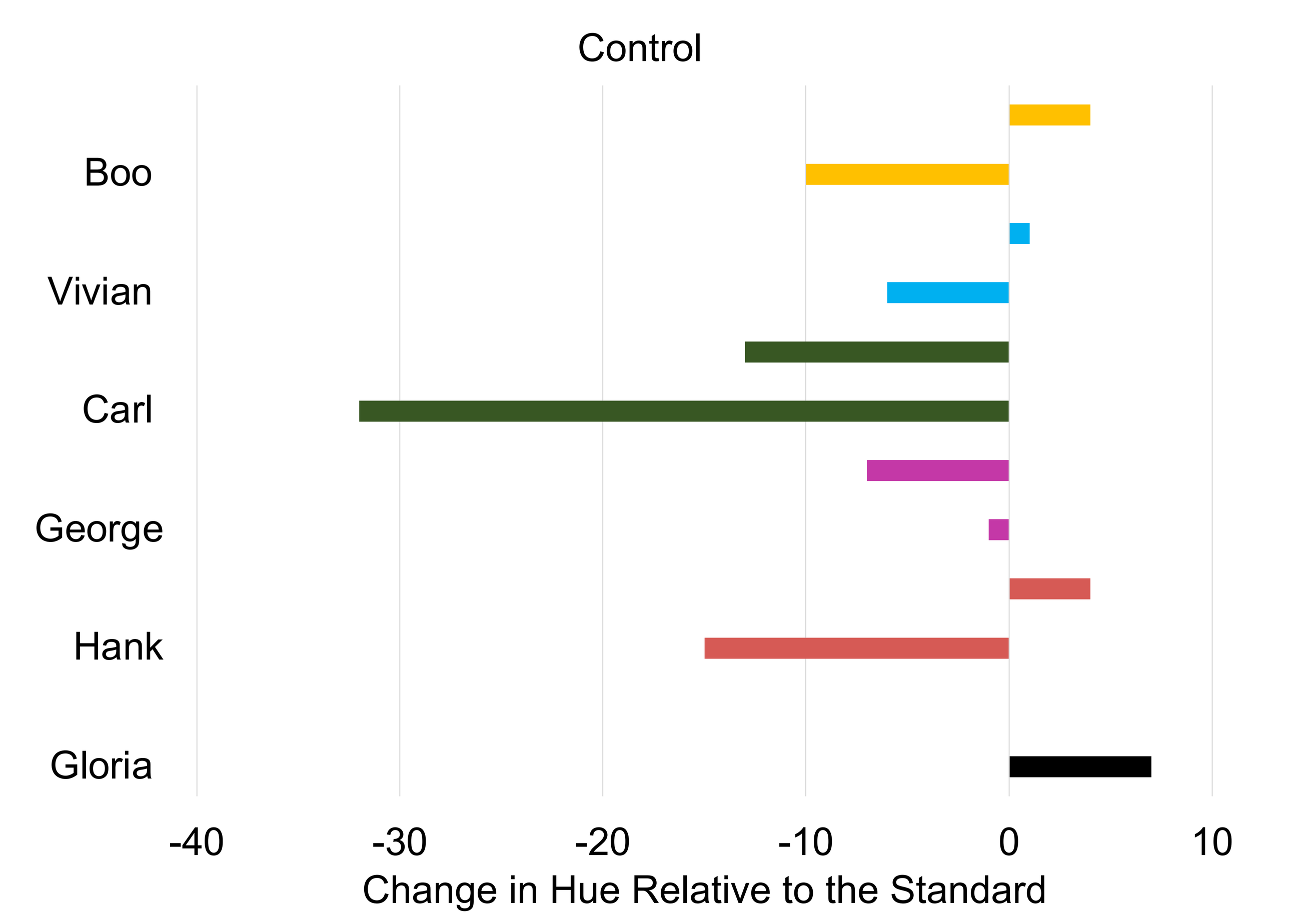
Figure 1. Example photo of Gloria's dorsal side with color standard

Figure 2. Example photo of Gloria's ventral side with color standard

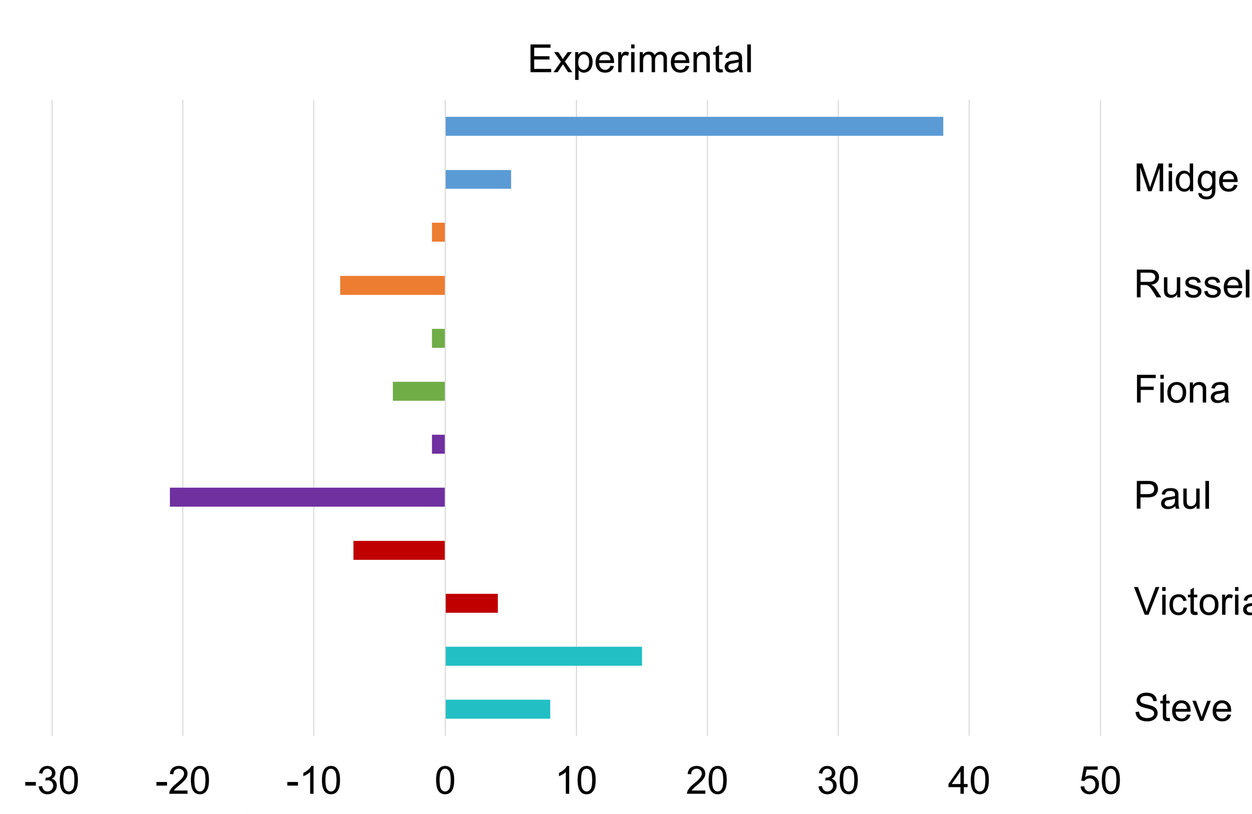
Results

The initial weighing of each salamander was high because it was conducted soon after a feeding while the rest of the weighing was done right before a feeding. Other than that, the weights of the salamanders have stayed relatively stable with the exception of one salamander.

From preliminary analysis, there was a lot of variation among both the control and the experimental groups. The experimental group averaged a change in hue by 2.25 +/- 14.42 while the control group averaged a change in hue of -5.67 +/- 10.87. I ran a t-test between the two groups of data and received a p value greater than 0.1. Three of the experimental salamanders experienced an increase in hue while four of the control salamanders experienced an increase in hue (Graphs 1 and 2). Two of the experimental salamanders experienced two notably larger increases in hue. The negative changes in hue outnumbered the positive.



Graph 1. Control group of salamanders. Each salamander on the left is associated with a color. The two bars represent the change in hue relative to the standard for the two spots analyzed. One of Gloria's spots is zero.



Graph 2. Experimental group of salamanders. Each salamander on the the right has a color associated with it. The two bars represent the change in hue relative to the standard for the two spots analyzed.

Discussion

Weight could be a factor to how well a salamander can allocate carotenoids to their pigmentation. One of the salamanders, Fiona, refused to eat for almost four weeks of feeding in a row which may reflect why she did not see an increase in hue.

The variation in the control could signify that there were other factors besides diet that affected yellow coloration. Despite attempts to control against morphological color change, it still may have occurred. Also sometimes when salamanders are frightened they will darken so when they were handled they may have darkened potentially causing the negative changes in hue. Glare from the lighting off of the salamander's skin also has caused difficulties in evaluating color change.

The variation in numbers also show that there is a lot of variety even among individual salamanders. Some areas on the salamanders did increase in yellow coloration and some decreased more towards brown coloration. If allocation of carotenoids did take place, they may be allocated differently in different areas of the body. The p value was greater than .1 indicating that there is no significant difference between the control and experimental group.

Diet may have played a role in color change but I can not currently conclude that the increases in yellow coloration were from diet alone.

Future Work

This poster only reflects preliminary analysis as this experiment is still ongoing. Currently, there is still two more weeks of feeding and data collection to be done. Because of the large amount of variation among just even the individual salamander, I would like to use Patternize, a R package, for easier comparison of both dorsal and ventral coloration (Van Belleghem et al 2017). I would also like to look at other values besides hue as potentially the saturation and brightness may have had an effect as well.

If possible, I would also like to analyze Rod's Food Krill+ and silverside fish to see exactly which types of carotenoids and how much are contained in each. I can then calculate how much of each was consumed by the salamanders.

Literature Cited

Bagnara J. T., Taylor J. D., and Hadley M. E. (1968) The Dermal Chromatophore Unit. *The Journal of Cell Biology*.**38**; 67-79.

Hill G. E. and Johnson J. D. (2012) The Vitamin A-Redox Hypothesis: A Biochemical Basis for Honest Signaling via Carotenoid Pigmentation. *The Americqn Naturalist*.**180**(5); E127-E150.

Kraemer A. C., Kissner J. and Adams D. C. (2012) Morphological Color-Change in the Red-Backed Salamander (*Plethodon cinereus*).*Copeia*.**4**; 748-755.

McGraw K. J. and Hardy L. S. (2006) Astaxanthin is responsible for the pink plumage flush in Franklin's and Ring-billed Gulls.*Journal of Field Ornithology*.**77**(1); 29-33.

Moriarty J. J. and Hall C. D. (2014) Amphibians and Reptiles in Minnesota. University of Minnesota Press, Minneapolis.

Reese R. W. (1969) The taxonomy and ecology of the tiger salamander (*Ambystoma tigrinum*) of Colorado. Ph. D. dissertation, University of Colorado, Boulder.

Rudh A. and Qvarnstrom A. (2013) Adaptive Coloration in Amphibians.*Seminars in Cells and Developmental Biology*.**24**; 553-561.

Semlitsch R. D. (2003) Amphibian Conservation. Smithsonian Institute, Washington D.C. Print.

Stuart S. N., Chanson J. S., Cox N. E., Young B. E., Rodrigues A. S. L., Fischman D. L., and Waller R. W. (2004) Status and Trends of Amphibian Declines and Extinctions Worldwide.*Science*.**306**; 1783-1786.

Van Belleghem S. M., Papa R., Ortiz-Zuazaga H., Hendrickx F., Jiggins C. D., McMillan W. O., and Counterman B. A. (2017) patternize: An R package for quantifying color pattern variation. *Methods in Ecology and Evolution.* **2017**; 1-9.

Vershinin A. (1996) Carotenoids in mollusca: approaching the functions.*Comp. Biochem. Physiol.***113B**(1); 63-71.

Waye H. L. (2013) Can a Tiger Change its Spots? A Test of the Stability of Spot Patterns for Identification of Individual Tiger Salamanders (*Ambystoma tigrinum*).*Herpetol Conserv Biol.***8**(2); 419-425

Tools used:
Photo editor: https://www169.lunapic.com/editor/
Image Color Summarizer: http://mkweb.bcgsc.ca/color-summarizer/?

Acknowledgements

I would like to thank UROP for funding this research project. I would also like to thank the University of Minnesota Morris and its faculty for shaping me to be the student I am today. I would also like to acknowledge my mentor Dr. Heather Waye. Lastly, I would like to thank my friends and family for their support.