

A test of an antipredatory function of conspicuous plastron coloration in hatchling turtles

Beth A. Reinke¹  · Ryan Calsbeek¹ · Devi Stuart-Fox²

Received: 27 November 2016 / Accepted: 28 February 2017 / Published online: 3 March 2017
© Springer International Publishing Switzerland 2017

Abstract Bright colorations in animals are sometimes an antipredatory signal meant to startle, warn, or deter a predator from consuming a prey organism. Freshwater turtle hatchlings of many species have bright ventral coloration with high internal contrast that may have an antipredator function. We used visual modeling and field experiments to test whether the plastron coloration of *Chrysemys picta* hatchlings deters predators. We found that bird predators can easily distinguish hatchling turtles from their backgrounds and can easily see color contrast within the plastron. Raccoons cannot easily discriminate within-plastron color contrast but can see hatchlings against common backgrounds. Despite this, we found that brightly-colored, high contrast, replica turtles were not attacked less than low contrast replica turtles, suggesting that the bright coloration is not likely to serve an antipredatory function in this context. We discuss the apparent lack of innate avoidance of orange coloration in freshwater turtles by predators and suggest that preference and avoidance of colors are context-dependent. Since the bright colors are likely not a signal, we hypothesize that the colors may be caused by pigments deposited in tissue from maternal reserves during development. In most species, these pigments fade ontogenetically but they may have important physiological functions in species that maintain the bright coloration throughout adulthood.

Keywords Antipredatory signal · Coloration · Visual model · *Chrysemys picta*

Electronic supplementary material The online version of this article (doi:10.1007/s10682-017-9892-5) contains supplementary material, which is available to authorized users.

✉ Beth A. Reinke
Elizabeth.a.reinke@dartmouth.edu

¹ Ecology and Evolutionary Biology, Dartmouth College, 78 College Street, Hanover, NH 03755, USA

² School of Biosciences, The University of Melbourne, Melbourne, VIC, Australia

Introduction

Conspicuous colors often serve as antipredatory signals to warn that an animal is unpalatable or unprofitable to consume (aposematism), to imitate an animal that is unpalatable or unprofitable (Batesian mimicry), to distract a predator's attention or confuse a predator (misdirection, shape distortion), or to startle a predator to give the prey a chance to escape (deimatic displays; Edmunds 1974). Bright colors are easy for predators to learn, difficult for them to forget, and are different enough from other prey that they cannot easily be confused (Joron 2003; Lindström et al. 1999).

Displays of conspicuous colors may involve a behavior that makes the prey organism look larger than it actually is (intimidation) or look like a larger animal (e.g. eyespot patterning) or may involve the sudden revelation of a bright coloration only upon disturbance or stress (startle display, flash display; Schlenoff 1985). The coloration can honestly signal the toxicity of the organism or may be dishonest signals meant to distract or overwhelm a predator from a palatable organism (Umbers et al. 2015). Without an associated display behavior, a bright color still could be anti-predatory if it is visible to the receiver and either an honest signal of the unprofitability of the prey, a mimic of a similar but aposematic organism, or if the predator has an innate avoidance of that color. To test whether a bright coloration is antipredatory, both the reception of the signal (the bright color) and the associated response of the receiver need to be measured since behavior should be at the interface of top-down and bottom-up approaches to any study of coloration (Kemp et al. 2015). However, studies investigating colorful displays often account for either signal reception or the response of the receiver, rather than both.

Many freshwater turtle hatchlings exhibit bright red, yellow, or orange colors on the ventral portion of the shell, called the plastron (Britson and Gutzke 1993). In most species, this coloration is lost in adults as the plastron takes on a more dull or solid hue (Britson and Gutzke 1993). However, it is not clear if the bright coloration in hatchlings is a conspicuous signal to predators. The small size, soft shell, and clumsy gait of hatchling turtles make them an easy prey item and they are consumed by a wide array of terrestrial and aquatic predators, including birds, raccoons, fish, spiders, and ants (Ernst and Lovich 2009). On land, the plastron is only visible if the hatchling turtle is turned on its back whereas in water, the color is only visible to a predator swimming below the turtle. Perhaps for this reason, most hatchling turtles prefer shallow water near the shoreline and do not venture into deep water (Ernst and Lovich 2009). Some research suggests that the bright ventral coloration may be an aposematic signal that warns of potentially detrimental behaviors (such as kicking and clawing) to an aquatic predator, the largemouth bass (Britson and Gutzke 1993), though manipulative experiments failed to support this theory (Britson 1996). Because the entirety of the plastron is often a bright color, it likely is not misdirecting the attention of a predator or obscuring the true shape of the turtle when viewed underneath. However, many terrestrial predators will pick up or flip a hatchling turtle before consuming it, revealing the previously concealed bright plastron coloration. Campbell and Evans (1972) suggested that the bright coloration of *Platysternon megacephalum* may serve as a 'flash' display. Thus, the coloration of hatchlings in many species may have evolved under the strong selective pressure of predation by startling predators and increasing the survival probability of a hatchling reaching the aquatic environment after emerging from the nest.

We use a freshwater turtle species, *Chrysemys picta*, (Schneider 1783) with a brightly colored plastron to investigate a possible antipredatory mechanism of plastron color by

combining visual modeling and behavioral field experiments. The painted turtle (*Chrysemys picta*) is a widespread, abundant North American turtle with a bright red–orange plastron, often with a black contrasting spot in the center (henceforth referred to as the plastral shape; Fig. 1). Painted turtle eggs are laid in the early summer and hatch in late summer. In some regions, hatchlings emerge from the nest and move to water immediately after hatching but in colder northern regions, hatchlings remain underground until the following spring. In either case, hatchlings are prone to predation while moving across land from their nesting site, which may be up to 600 m from water (Ernst and Lovich 2009). We calculated the contrasts of *C. picta* hatchling turtles against their nesting sites, and the contrasts of the bright orange against the plastral shape, to determine whether the bright coloration and high contrast of *C. picta* appears conspicuous to common predators such as raccoons (*Procyon lotor*), crows (Family: Corvidae), gulls (Family: Laridae), and herons (Family: Ardeidae). Then, using life-like replicas with similar reflectance properties, we collected data on attack frequency and severity on variants of these plastron colors to determine whether the coloration in *C. picta* is likely to be an antipredatory mechanism and to determine the relative importance of contrast within the plastron and against the background.

Fig. 1 Ventral views of the plastron of two painted turtle *Chrysemys picta* hatchlings. Arrows point to approximate areas where reflectance spectra were collected. Scale bar 20 mm



Methods

Animal collection

Chrysemys picta hatchlings were either collected near Musky Bay, Lac Courte Oreilles in Sawyer County, Wisconsin, in May as they emerged from their nests ($n = 3$) or obtained commercially from Florida Herpetology, Inc ($n = 5$) to collect reflectance spectra and to measure righting responses. One individual was found dead of unknown causes and was used for creating molds. It is unknown if any individuals came from the same clutch. All animal collection and use was approved by the Wisconsin Department of Natural Resources and the Dartmouth College Institutional Animal Care and Use Committee (Protocol #cals.rg.3).

Reflectance spectra collection

Reflectance spectra were collected using an Ocean Optics Jaz spectrometer with an inbuilt PX2 light source and bifurcated fiber optic probe fitted with a custom probe holder with the angle of illumination and reflection at 45° relative to the collection surface. Measurements were taken relative to a thick layer of barium sulfate as a 99% reflectance standard (Grum and Luckey 1968). All individuals were dried with a towel before collecting spectra. Reflectance spectra were collected at two points on orange and black parts of the right or left abdominal scute on the plastron (Figs. 1, 2). On the carapace, a reflectance spectrum was collected on the second right and left costal scute and these were later averaged (Fig. 2). Background reflectance spectra were collected in the areas adjacent to the catch sites on bare sandy patches and green vegetation (Fig. 2). Two spectra of each type were collected and averaged. Background reflectances are likely biologically relevant because their positions relative to catch sites and known nesting sites necessitate that emerging hatchlings must cross these areas to reach water. All spectral data were averaged over 5 nm intervals.

Spectral sensitivity derivations

Raccoons (*Procyon lotor*), crows (Corvidae), gulls (Laridae), and herons (Ardeidae), are known predators of *C. picta* hatchlings (Ernst and Lovich 2009) and are common throughout most of *C. picta*'s range. Raccoons are monochromats (essentially color blind) while birds are tetrachromats. We used the λ_{\max} (wavelength of maximum response) of raccoons from Jacobs and Deegan (1992) and reconstructed the spectral sensitivity equation using formulae in Govardovskii et al. (2000) for A1 chromophores. The transmission of raccoon ocular medium has not been determined but we used the 50% transmission cut-off of the golden hamster, a nocturnal mammal with some diurnal foraging activity, to approximate the raccoon ocular media transmission (Brainard et al. 1994). The final spectral sensitivity function for the raccoon had a $\lambda_{\max} = 560$ nm. The three bird families we were interested in (Corvidae, Laridae, and Ardeidae) belong to three separate orders (Passeriformes, Charadriiformes, and Pelicaniformes, respectively). Because avian spectral sensitivity is highly conserved within the two groups of vision (ultraviolet-sensitive [UVS] and violet-sensitive [VS]), we used available average spectral sensitivities for UVS (λ_{\max} ultraviolet sensitive cone = 370 nm, λ_{\max} short wavelength sensitive cone = 455 nm, λ_{\max} medium wavelength sensitive cone = 540 nm, λ_{\max} long wavelength

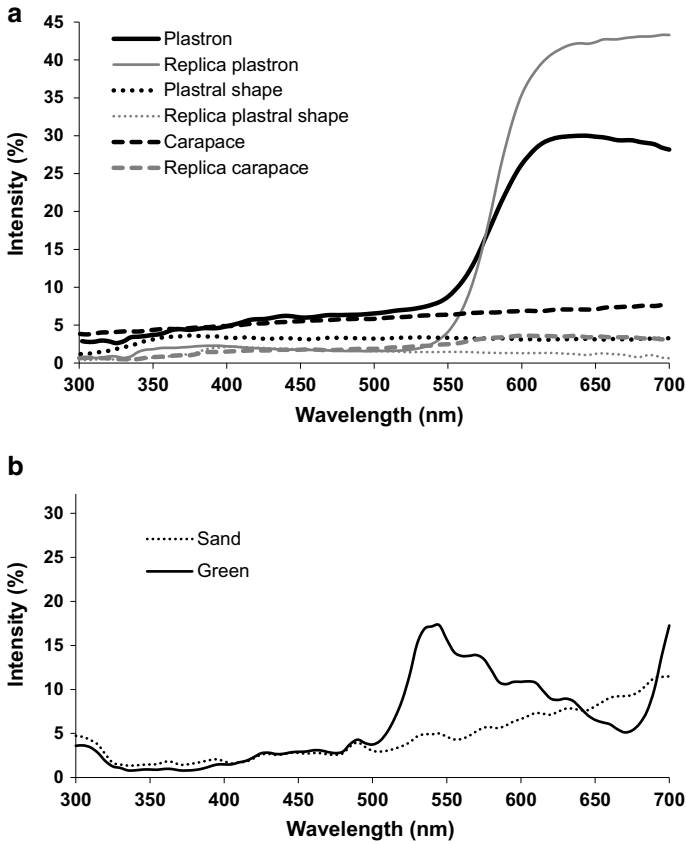


Fig. 2 **a** Reflectance spectra of a representative *Chrysemys picta* hatchling (black lines) and a replica *Chrysemys picta* hatchling (grey lines). **b** Background reflectance spectra near nesting sites. Plastron and carapace spectra are each the average of two points collected on an individual. Background spectra are sand and green averages collected from several points

sensitive cone = 610 nm, λ_{\max} double cone = 560 nm) and VS (λ_{\max} ultraviolet sensitive cone = 415 nm, λ_{\max} short wavelength sensitive cone = 475 nm, λ_{\max} medium wavelength sensitive cone = 540 nm, λ_{\max} long wavelength sensitive cone = 605 nm, λ_{\max} double cone = 560 nm) birds (Endler and Mielke 2005). Birds in Laridae are ultraviolet-sensitive while birds in Corvidae and Ardeidae are violet-sensitive (Hunt et al. 2009; Ödeen et al. 2010).

Visual modeling

We first calculated receptor quantum catches Q for each receptor type i for each class of predators (raccoons, UVS birds and VS birds):

$$Q_i = \int R_i(\lambda)S(\lambda)I(\lambda)d(\lambda) \tag{1}$$

where R is the spectral sensitivity of the photoreceptor, S is the reflectance of the color patch, and I is the irradiance. Painted turtle nests occur in many degrees of shade and

vegetation cover so we used irradiance spectra representative of forest edge from Stuart-Fox et al. (2007), but also modelled the perceived colors under edge and full shade and presented these results as supplementary material (Weisrock and Janzen 1999; Tables S1–S2). We applied the von Kries transformation to normalize the quantum catch of the receptor to the background light environment (Eqs. 1A and 1B).

$$k = 1 / \int R_i(\lambda) I(\lambda) d(\lambda) \tag{1A}$$

$$q = kQ \tag{1B}$$

We then applied the receptor noise limited (RNL) model of color discrimination (Vorobyev and Osorio 1998) to estimate the contrast ΔS between plastron and background colors in units of ‘just noticeable differences’ (JNDs), whereby a JND of 1 is the threshold at which, under ideal conditions, two colors are easily discriminable. Values less than 1 are not discriminable, and values greater than 1 get increasingly discriminable. For a monochromat, achromatic contrast is calculated as

$$\Delta L = \ln(\Delta f) / w \tag{2}$$

and for tetrachromats, chromatic contrast is

$$\Delta S = \frac{1}{w} \sqrt{\frac{(w_1 w_2)^2 (\Delta f_4 - \Delta f_3)^2 + (w_1 w_3)^2 (\Delta f_4 - \Delta f_2)^2 + (w_1 w_4)^2 (\Delta f_3 - \Delta f_2)^2 + (w_2 w_3)^2 (\Delta f_4 - \Delta f_1)^2 + (w_2 w_4)^2 (\Delta f_3 - \Delta f_1)^2 + (w_3 w_4)^2 (\Delta f_2 - \Delta f_1)^2}{(w_1 w_2 w_3)^2 + (w_1 w_2 w_4)^2 + (w_1 w_3 w_4)^2 + (w_2 w_3 w_4)^2}} \tag{3}$$

where w is a measure of photoreceptor noise within the receptor field known as the Weber fraction, the receptor signal (f) is proportional to the natural logarithm of the quantum catch: $f_i = \ln q_i$, and Δf_i is

$$\ln q_{i(\text{spec1})} - \ln q_{i(\text{spec2})} = \ln \left(\frac{q_{i(\text{spec1})}}{q_{i(\text{spec2})}} \right). \tag{4}$$

Achromatic contrasts for birds were calculated using Eq. 2 above and spectral sensitivities for the LWS photoreceptor within the double cones. Because photoreceptor noise values are not available for raccoons, we used $w = 0.03$, $w = 0.05$, and $w = 0.07$. We found no qualitative difference between the ΔS for different Weber fractions and so only report the results for $w = 0.05$. For the avian predators, relative photoreceptor abundances were obtained from Hart (2001) and averaged across species within each order of interest (Passeriformes, Charadriiformes, and Pelicaniformes) to calculate the Weber fractions with the assumption that $w(L) = 0.06$ as in Olsson et al. (2015; Table 1). The Weber fraction for achromatic contrast calculations for birds was assumed to be $w = 0.05$. All tetrachromatic visual modeling was performed in R (3.2.4) with the package ‘pavo’ (Maia et al. 2013).

Righting responses

Righting responses were measured to determine if individual turtles would have time to escape after a predator is startled by the appearance of a bright color. We measured righting responses as the time it takes for the *C. picta* hatchling to flip from a position on

Table 1 Weber fractions and associated cone proportions for UV, short, medium, and long wavelength-sensitive receptors in Passeriformes, Charadriiformes, and Pelicaniformes

	w(U)	w(S)	w(M)	w(L) ^a
Charadriiformes	0.186	0.106	0.087	0.060
Cone proportions	1	3.1	4.6	9.6
Passeriformes	0.188	0.140	0.114	0.060
Cone proportions	1	1.8	2.7	9.8
Pelicaniformes	0.208	0.134	0.086	0.060
Cone proportions	1	2.4	5.8	12

^a W(L) is assumed to equal 0.06 based on Olsson et al. (2015)

the carapace back to the plastron at 26°C. There is a slight keel in hatchlings that prohibits a flat dorsal orientation. The righting responses of all live individuals ($n = 8$) were measured three times, on alternate sides of the carapace, on a smooth surface indoors to control temperature. We did not measure running speed since the slow, clumsy gait of hatchling turtles makes speed unlikely to be the determining factor in whether a predator will consume an individual.

Turtle replicas

A dead *C. picta* hatchling was used to make the mold for the experimental models by pressing the carapace into a ball of Pluffy clay (Sculpey Polyform Products Company, mixed with vegetable oil) and then removing it. The plastron was molded similarly with a shallow imprint. Then, a clay replica of the carapace and plastron was made using that mold and these were used to make durable molds out of silicone (Pinkysil). We used polyurethane (Dalchem, DC202 Rapid Set Polyurethane Resin) to make the final replicas. Acrylic paints were mixed and tested on dried polyurethane until the reflectance did not differ from the reflectance spectra collected from live individuals by more than 15% intensity at any wavelength. Three colors were made: an orange to match the plastron, a black to match the plastral shape, and a brown to match the carapace (Fig. 2). All paint

Fig. 3 Paint chips and possible beak damage on a model of a *Chrysemys picta* hatchling. This damage would be ranked a “2”



colors chipped easily off the polyurethane (i.e. with the tap of a fingernail) meaning predation attempts could be easily visualized (Fig. 3).

Predation experiments

To determine if the plastral shape contrast is a necessary component of the antipredatory function, we created four types of replicas. The control resembled an actual *C. picta* hatchling with an orange plastron and black plastral shape (high contrast within shell and with background). One treatment group was orange with no plastral shape (high contrast with background), another group had an unpainted plastron that appears tan with no plastral shape (low contrast within shell), and finally, there was an unpainted plastron with a black plastral shape group (high contrast within shell; Fig. 4). From mid-April to mid-May hatchlings typically emerge from nests in the northeastern United States. Along each of five transects, we placed five models from each treatment group ($n = 20$ per transect) between lakes with known turtle populations and known or likely nesting sites ($n = 5$). Replicas were placed approximately 1 m from the waterline and at least 1 m from each other and the order of the treatments was randomized. For Trial 1, all replicas were either placed dorsal-side up or ventral-side up for two days. For the second two days (Trial 2), all



Fig. 4 From *top-left* clockwise control *C. picta* replica with high contrast within shell and with background, orange treatment with high contrast with background only, unpainted plastron with low contrast within shell and with background, unpainted plastron with plastral shape for high contrast within shell only

replicas were flipped (i.e. dorsal-side up were changed to ventral-side up and vice versa). We tested both orientations to determine the frequency at which replicas were turned over ventrally, as well as to measure the likelihood of predation attempts when an individual was already exposed ventrally and vulnerable. Each replica was placed at a random angle relative to the shoreline and recorded so that any movement with minimal or ambiguous damage could be verified as a predation attempt. Movement and predation attempts were recorded and reset daily with repairs made as necessary. Predation attempts were scored from 0 to 4 with 0 being no change, 1 being a rotation or movement but no obvious damage, 2 meaning rotation and damage, 3 meaning the model was flipped, and 4 meaning the replica was taken or could not be found (see Fig. 3 for example of damage ranked “2”).

All statistical analyses were performed in JMP (SAS 12.0.0). For all models, we included site and transect nested within site as random effects. First, we tested if the probability of being attacked varied depending on the orientation of the replica (dorsal or ventral-side up). We calculated the difference in attack probability between orientations and tested whether the mean of the distribution of these differences differed from zero using a one-sample *t* test. Then, to determine if the type of replica predicted predation, we performed two separate generalized linear models. The first used attack as a binary variable with a binomial distribution and the second used the severity of attack (0–4 as described above) with a Poisson distribution and both used the type of replica as a predictor variable.

Results

Visual modeling

Visual models suggest that raccoons can discriminate painted turtle hatchlings from their backgrounds ($\Delta L > 1$, Table 2), with the lowest contrast between the orange color and the sand background ($\Delta L \sim 2$). Raccoons cannot easily discriminate the black plastral shape from the orange color in edge habitat ($\Delta L < 2$, Table 2). Both the chromatic and achromatic contrasts we calculated suggest that a hatchling turtle is easily discriminable ($\Delta S = 2.5\text{--}8$; $\Delta L = 2.5\text{--}10.5$) by all three types of birds against all backgrounds (Tables 2, 3). In terms of achromatic contrasts, hatchlings were more easily discriminable to birds than they were to raccoons (Table 2). As expected, the plastron contrasted more

Table 2 Achromatic contrasts (ΔL) of hatchling turtles against two backgrounds and within the plastron in shade showing means ($n = 8$) with standard deviation in parentheses as seen by raccoons and three orders of birds

	Sand		Vegetation		Plastron
	Carapace	Orange plastron	Carapace	Orange plastron	Plastral shape
Raccoon	3.80 (2.93)	1.80 (0.83)	3.84 (2.93)	6.37 (1.59)	1.57 (1.21)
Charadriiformes	5.04 (2.92)	9.16 (3.56)	9.80 (4.94)	2.92 (1.05)	6.22 (3.30)
Passeriformes	5.04 (2.42)	9.16 (3.56)	9.80 (4.94)	2.92 (1.05)	6.22 (3.30)
Pelicaniformes	5.04 (2.42)	9.16 (3.56)	9.80 (4.94)	2.92 (1.05)	6.22 (3.30)

Bolded values are those which are below or near the point where discrimination is possible environment ($\Delta L < 1$). In most cases, birds can more easily discriminate hatchling turtles against their backgrounds than raccoons

Table 3 Chromatic contrasts (ΔS) of hatchling turtles against two backgrounds and within the plastron in shade, edge, and sun (respectively) showing means ($n = 8$) with standard deviation in parentheses

	Sand		Vegetation		Plastron
	Carapace	Orange plastron	Carapace	Orange plastron	Orange against black
Gull	4.79 (3.12)	4.85 (1.21)	6.57 (1.99)	6.99 (2.14)	7.20 (2.31)
Crow	2.98 (1.12)	3.07 (1.32)	4.16 (1.73)	5.19 (2.35)	5.76 (2.10)
Heron	3.17 (1.34)	3.20 (1.32)	4.48 (1.92)	6.23 (2.69)	5.91 (2.12)

with the background than did the carapace. Birds can easily discriminate the black shape on the plastron from the orange, meaning the black plastral shape provides a high contrast that makes the plastron conspicuous to some predators but not others.

Righting responses

Six *C. picta* exhibited righting responses. Average righting response times ranged from 14.34 to 69.27 s with the longest response time at 129.7 s. Two individuals never moved during their three 1-min trials and so their times are not included in the listed averages.

Predation experiments

Including both days of the trial, 64 of the 943 replicas oriented dorsal-side up were attacked (scored ≥ 1). Of the 923 replicas oriented ventral-side up, 58 were attacked (scored ≥ 1); 14 attacks on the orange treatment, 10 on the orange with plastral shape treatment, 13 on the tan treatment, and 21 on the tan with plastral shape treatment. We found that the likelihood of attack was no different if the replica was oriented first dorsally or ventrally in either trial ($t = -1.04$, $df = 478$, $P = 0.298$). A majority of the attacks could not be attributed to a specific predator because of non-specific marks or movement, but some were obvious beak marks (Fig. 3) and in one case, the replica was moved several meters and torn apart, likely by a raccoon. To determine if there was an effect of treatment, we pooled data from both trials for remaining analyses and only analyzed replicas oriented ventral side-up. We found that we could not reject the null hypothesis that all types of replicas are attacked with equal probability when oriented ventral side-up (GLM $\chi^2 = 2.13$, $df = 3$, $P = 0.55$). We also found that we could not reject the null hypothesis that all replicas were attacked with equal severity (GLM $\chi^2 = 5.28$, $df = 3$, $P = 0.15$; Fig. 5).

Discussion

Though the visual modeling results suggest that many predators can see *C. picta* plastrons with a high level of contrast against the background and within the plastron pattern, field experiments suggest that bright coloration is unlikely to be an antipredatory mechanism. The long righting response times of hatchling individuals show that predators that flip hatchling turtles would likely have ample time to observe and react to the color, but flipped hatchling replicas with bright colors and high contrasts were not attacked significantly less

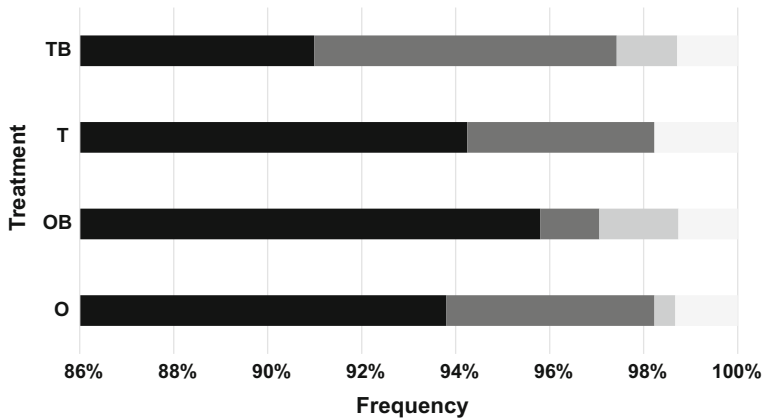


Fig. 5 Frequencies of types of attack on replicas of *Chrysemys picta* hatchlings of each treatment when oriented ventral-side up. TB is tan with a black plastral shape, T is tan with no plastral shape, OB is orange with a black plastral shape (natural coloration), and O is orange with no plastral shape. *Black* is no attack, *dark grey* is an attack severity of 1, *grey* is an attack severity of 3, and *light grey* is an attack severity of 4. No attacks of severity 2 occurred when replicas were ventral-side up. Replicas were repaired daily and data show the results of five transects of 20 individuals at five sites across 4 days

than hatchling replicas with dull colors and low contrasts. Additionally, only 34% of the replicas oriented dorsal side-up that were moved at all were flipped, meaning that predators would likely only see the ventral coloration less than half the time.

These results suggest that predators do not have an innate aversion to orange coloration in freshwater turtles, with low or high contrast. Though predators may exhibit an unlearned avoidance of red or orange colors, presumably because of aposematic prey, an innate preference for red and orange colors still exists in some situations since red often indicates ripeness of fruits (Schmidt and Schaefer 2004). In some cases, there is no innate avoidance or preference for typically aposematic colors until those behaviors are learned (e.g. Ham et al. 2006) and in the wild, the degree of neophobia can vary widely by individual (Marples et al. 1998). Though many bird predators exhibit neophobia and dietary conservatism when first encountering novel prey (Marples and Kelly 1999; Marples et al. 1998), bright coloration in hatchling turtles is fairly common, so it is unlikely to be a major deterrent. Thus, the response to red or orange coloration is context-dependent and without an associated toxin or otherwise noxious behavior, bright colorations may make a palatable organism just as likely to be attacked as a dull coloration if they are not rare. For example, Gamberale-Stille and Tullberg (2001) found that naïve birds did not have a preference when presented with red or green fruits, but did avoid red when presented with red or green insects, regardless of movement. Since hatchling turtles have no known defense, it is likely that predators have never learned avoidance behaviors and so do not consider turtles unpalatable or unprofitable to consume. This is the first case, to our knowledge, of a lack of preference-avoidance for a bright coloration in a palatable vertebrate prey item.

Our results, in conjunction with a previous experiment using an aquatic predator (Britson 1996), suggest that the bright plastron coloration of hatchling freshwater turtles does not likely have an antipredatory signaling function. For a signal to be effective, it must be readily transmitted and received within the environment in which it occurs (Endler 1992). We attempted to determine if plastron coloration could deter predators but did not

find that predators attacked or avoided brightly colored replicas any more or less than low contrast replicas, despite being able to discriminate them easily from their backgrounds. Additionally, hatchling turtles have no known behavior to make the ventral coloration obvious. Because of the ventral location of plastron coloration, it is only visible while the turtle is flipped on its dorsal side by a predator while on land, or to another organism from underneath while the turtle is swimming in the water column. In shallow freshwater environments, before the water column gets deep enough to filter much light, red and orange are typically conspicuous against the green background. If plastron coloration in freshwater turtles is an antipredatory signal, the red, orange, and yellow colorations are the ideal wavelengths to be transmitted and received in shallow freshwater environments. However, previous experiments have suggested that the coloration is not likely to be aposematic or have any detrimental effects on aquatic predators (Britson 1996), meaning the coloration should be detrimental to an individual by making it more conspicuous with no defense. The coloration is also not likely to mimic a dangerous animal or plant because of the diversity of bright colors and patterns across species. Thus it is possible that the bright coloration is not a signal at all, but a byproduct of some other physiological function.

An alternative explanation of bright coloration in hatchling turtles is that the color may be caused by leftover pigments obtained from maternal reserves in the yolk. Carotenoids, for example, cannot be synthesized *de novo* by animals but may be obtained through maternal reserves and are common pigments in eggs (McGraw et al. 2005). It is commonly thought that carotenoids are allocated to eggs to give the developing offspring antioxidant benefits (Blount et al. 2002; Haq et al. 1996) and in fact, because embryonic development is a time of high oxidative damage, antioxidants are especially important during this stage (Costantini et al. 2008). We have confirmed that carotenoids are present in the plastron of *C. picta* (Reinke, unpublished data).

Many pigments, including carotenoids, can have physiological functions as antioxidants and immune-boosters and so may have a functional significance during development in the egg (Karadas et al. 2005). However, carotenoids also have the potential to become dangerous pro-oxidants (El-Agamey et al. 2004) so excess pigments could be deposited into the shell because the ventral location makes the conspicuous color less likely to be seen and the external location is unlikely to do significant pro-oxidant harm until the pigments degrade and fade ontogenetically. This hypothesis could also explain why some freshwater turtle species maintain a bright coloration. For instance, *Chrysemys picta* are one of the few freshwater species that maintain a bright coloration throughout adulthood but only the northernmost subspecies do so. These subspecies are also freeze- and anoxia-tolerant, processes that require high levels of antioxidants to combat oxidative stress during recovery. Carotenoids may act as necessary antioxidants during the winter months, and may be stored, with no signal function, in the relatively secreted ventral location during the warmer months. Further research into freshwater turtle coloration needs to be undertaken to fully understand any functional significance of the coloration, and especially to understand any physiological function the pigments may have. However, this study shows that bright ventral coloration is unlikely to have an antipredatory function and thus is unlikely to be a signal.

Acknowledgements The authors would like to thank Rebecca and Rick Nelson, Gary and Linda Pulford, and Willem Roosenberg and his field team for their significant contributions to hatchling collection efforts. They would also like to thank T. Nelson, J. Murphy, M. Pardue, M. C. Duryea, N. Hess, A. Edsor, K. Smith, and A. Formby. This project was funded by a National Science Foundation East Asia and Pacific Summer Institute Fellowship Grant (#1414687) in partnership with the Australian Academy of Sciences.

References

- Blount J, Surai P, Houston D, Møller A (2002) Patterns of yolk enrichment with dietary carotenoids in gulls: the roles of pigment acquisition and utilization. *Funct Ecol* 16:445–453
- Brainard G, Barker F, Hoffman R, Stetson M, Hanifin J, Podolin P, Rollag M (1994) Ultraviolet regulation of neuroendocrine and circadian physiology in rodents. *Vis Res* 34:1521–1533
- Britson CA (1996) Predatory responses of largemouth bass (*Micropterus salmoides*) to conspicuous and cryptic hatchling turtles: comparative and manipulative experiments. In: Behavioral defenses associated with plastral coloration in hatchling, freshwater turtles. University of Memphis, unpublished dissertation
- Britson CA, Gutzke WH (1993) Antipredator mechanisms of hatchling freshwater turtles. *Copeia* 1993:435–440
- Campbell HW, Evans WE (1972) Observations on the vocal behavior of chelonians. *Herpetologica* 28:277–280
- Costantini D, Fanfani A, Dell’Omo G (2008) Effects of corticosteroids on oxidative damage and circulating carotenoids in captive adult kestrels (*Falco tinnunculus*). *J Comp Physiol B* 178:829–835
- Edmunds M (1974) Defence in animals: a survey of anti-predator defences. Longman Publishing Group, London
- El-Agamey A, Lowe GM, McGarvey DJ, Mortensen A, Phillip DM, Truscott TG, Young AJ (2004) Carotenoid radical chemistry and antioxidant/pro-oxidant properties. *Arch Biochem Biophys* 430:37–48
- Endler JA (1992) Signals, signal conditions, and the direction of evolution. *Am Nat* 139:S125–S153
- Endler JA, Mielke PW (2005) Comparing entire colour patterns as birds see them. *Biol J Linn Soc* 86:405–431
- Ernst CH, Lovich JE (2009) Turtles of the United States and Canada. JHU Press, Baltimore, Maryland
- Gamberale-Stille G, Tullberg BS (2001) Fruit or aposematic insect? Context-dependent colour preferences in domestic chicks. *Proc R Soc Lond B Biol Sci* 268:2525–2529
- Govardovskii VI, Fyhrquist N, Reuter T, Kuzmin DG, Donner K (2000) In search of the visual pigment template. *Vis Neurosci* 17:509–528
- Grum F, Luckey GW (1968) Optical sphere paint and a working standard of reflectance. *Appl Opt* 7:2289–2294. doi:10.1364/ao.7.002289
- Ham A, Ihalainen E, Lindström L, Mappes J (2006) Does colour matter? The importance of colour in avoidance learning, memorability and generalisation. *Behav Ecol Sociobiol* 60:482–491
- Haq A-U, Bailey CA, Chinnah A (1996) Effect of β -carotene, canthaxanthin, lutein, and vitamin E on neonatal immunity of chicks when supplemented in the broiler breeder diets. *Poult Sci* 75:1092–1097
- Hart NS (2001) Variations in cone photoreceptor abundance and the visual ecology of birds. *J Comp Physiol A* 187:685–697
- Hunt DM, Carvalho LS, Cowing JA, Davies WL (2009) Evolution and spectral tuning of visual pigments in birds and mammals. *Philos Trans R Soc B Biol Sci* 364:2941–2955
- Jacobs GH, Deegan JF II (1992) Cone photopigments in nocturnal and diurnal procyonids. *J Comp Physiol A* 171:351–358
- Joron M (2003) Aposematic coloration. In: Encyclopedia of insects. Academic Press, Elsevier Inc., Burlington, MA, pp 39–45
- Karadas F, Pappas AC, Surai PF, Speake BK (2005) Embryonic development within carotenoid-enriched eggs influences the post-hatch carotenoid status of the chicken. *Comp Biochem Physiol B Biochem Mol Biol* 141:244–251
- Kemp DJ, Herberstein ME, Fleishman LJ, Endler JA, Bennett ATD, Dyer AG, Hart NS, Marshall J, Whiting MJ (2015) An integrative framework for the appraisal of coloration in nature. *Am Nat* 185:705–724. doi:10.1086/681021
- Lindström L, Alatalo RV, Mappes J (1999) Reactions of hand-reared and wild-caught predators toward warningly colored, gregarious, and conspicuous prey. *Behav Ecol* 10:317–322
- Maia R, Eliason CM, Bitton PP, Doucet SM, Shawkey MD (2013) pavo: an R package for the analysis, visualization and organization of spectral data. *Methods Ecol Evol* 4:906–913
- Marples N, Kelly D (1999) Neophobia and dietary conservatism: two distinct processes? *Evol Ecol* 13:641–653
- Marples NM, Roper TJ, Harper DG (1998) Responses of wild birds to novel prey: evidence of dietary conservatism. *Oikos* 83:161–165
- McGraw K, Adkins-Regan E, Parker R (2005) Maternally derived carotenoid pigments affect offspring survival, sex ratio, and sexual attractiveness in a colorful songbird. *Naturwissenschaften* 92:375–380

- Ödeen A, Håstad O, Alström P (2010) Evolution of ultraviolet vision in shorebirds (Charadriiformes). *Biol Lett* 6:370–374
- Olsson P, Lind O, Kelber A (2015) Bird colour vision: behavioural thresholds reveal receptor noise. *J Exp Biol* 218:184–193
- Schlenoff DH (1985) The startle responses of blue jays to *Catocala* (Lepidoptera: Noctuidae) prey models. *Anim Behav* 33:1057–1067
- Schmidt V, Schaefer HM (2004) Unlearned preference for red may facilitate recognition of palatable food in young omnivorous birds. *Evol Ecol Res* 6:919–925
- Schneider JG (1783) *Allgemeine Naturgeschichte der Schildkröten: nebst einem systematischen Verzeichnisse der einzelnen Arten und zwei Kupfren*. J.G. Müller, Leipzig
- Stuart-Fox D, Moussalli A, Whiting MJ (2007) Natural selection on social signals: signal efficacy and the evolution of chameleon display coloration. *Am Nat* 170:916–930
- Umbers KD, Lehtonen J, Mappes J (2015) Deimatic displays. *Curr Biol* 25:R58–R59
- Vorobyev M, Osorio D (1998) Receptor noise as a determinant of colour thresholds. *Proc R Soc Lond Ser B Biol Sci* 265:351–358
- Weisrock D, Janzen F (1999) Thermal and fitness-related consequences of nest location in painted turtles (*Chrysemys picta*). *Funct Ecol* 13:94–101