

Is coloration linked to parasite load in freshwater turtles?

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Abstract:

The Hamilton and Zuk hypothesis suggests a causal link between parasite load and the development of epigamic characters, such that the best mate is the one with the most conspicuous features combined with fewest parasites. Based on the assumption that they are good indicators of individual ability, I tested if the color of the Painted Turtle's (*Chrysemys picta*) chin and the length of their claws are affected by their endoparasite load. I predicted that (1) individuals with fewer endoparasites should have more intense chin coloration as predicted by the Hamilton and Zuk hypothesis and that (2) males with fewer endoparasites should have longer claws. To assess the degree of infection, I screened tainted blood smears for blood parasites. I also quantified the turtle's chin color intensity after linearizing and equalizing digital photographs. I found no significant predictors of the chin's color intensity and thus no support to the Hamilton and Zuk hypothesis in the Painted Turtle. I also conclude that it is unlikely that the bright colors of the Painted turtle are sex-linked or used in signaling. As for the relationship between the claw length and the endoparasite load, neither the parasite load, the body index nor the carapace length were significant predictors of claws length. This study may be considered as an initial step to quantify variation of color production prior to UV variations and more research with stronger designs is needed to determine the effect of blood parasites on the Painted turtle physiology, development and appearance.

Keywords: Hamilton and Zuk hypothesis, sexual selection, painted turtle, hemiparasites, digital photography

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Introduction:

Darwin's theory of evolution by natural selection is well known and has been studied for over a century. However, Darwin's theory of sexual selection started to largely draw the attention of scientists in the 1970's causing an explosion of research in that field of study (Hosken and House, 2011). Darwin defined sexual selection as "the advantage which certain individuals have over other individuals of the same sex and species solely in respect of reproduction". It differs from natural selection because the most conspicuous and impressive traits selected by sexual selection are not necessarily selected by natural selection: sexual selection results from reproductive success variation (choice and competition for mates) while natural selection results from differences in all other fitness elements, most often survival (Price, 1998; Hosken and House, 2011). Natural and sexual selection can still both have an effect on the same trait. Whereas sexual selection describes intra-specific reproductive competition, color marking is thought to be a signal for inter and intra-specific recognition and to play a role when searching for the right sexual mate (Liu *et al.*, 2013). For Gelada primates, it has been demonstrated that the redness of the chest varies as a function of age, and may be considered as a secondary sexual character associated with sexual maturation or reproduction (Bergman and Beehner, 2008). Another study that used a phylogenetic comparative approach on the Agamidae family (Australian dragon lizard) suggested that the increase over time of their color patterns used as an ornament is driven by sexual selection instead of natural selection (Chen *et al.*, 2012).

The Hamilton and Zuk hypothesis suggests a causal link between parasite load and the development of epigamic characters, such that the best mate is the one with the most conspicuous

features combined with fewest parasites (Hamilton and Zuk, 1982). Therefore, within a population, individuals with brighter colors (which are considered secondary sexual ornaments) should also have fewer parasites (Hamilton and Zuk, 1982; Martin and Johnsen, 2007a). Only the fittest individuals can afford to produce these bright colors, either because they can acquire more energy, or they are the only ones able to survive while conspicuous to predators. Female Satin Bower birds (*Ptilonorhynchus violaceus*) use UV coloration as an indicator of health when searching for a potential mate. UV coloration is a good indicator of individual quality because it predicts the intensity of blood parasite infection (brightest plumage is associated with the lowest degree of infection) (Doucet and Montgomerie, 2003). However, a recent study found that age could be a more trustworthy signal of male warbler quality because young uninfected and well-ornamented males could simply have never encountered parasites and do not necessarily have parasite-resistant genes (Freeman and Conor, 2018).

Although the Hamilton and Zuk hypothesis has been studied thoroughly with many bird species [passerines (Read, 1987) and barn swallows (Møller, 1990)], I found no tests of this hypothesis with respect to the importance of coloration as a sexual selection trait in freshwater turtles. Studies over a wide range of taxa have both delivered support and lack of support for the Hamilton and Zuk hypothesis. Therefore, it is important to study multiple host-parasite systems before drawing general conclusions (Hamilton and Poulin, 1997). Some studies have been conducted in reptiles, but mostly with lizards. Some species with male and female alike coloration can still use intra-sexual communication to distinguish the sex of each other, but the mate choice is often based on additional features more female or male-like rather than solely on coloration (Svensson *et al.*, 2009; Swierk and Langkilde, 2013). Therefore, coloration could still

be an indicator of health, but other features such as size or other chemical signals could also have an impact when choosing mates. A study on the Common Wall lizard (*Podarcis muralis*) indicated that less parasitized individuals showed both brighter ventral yellow color and femoral secretions of costly compounds, providing evidence that parasite load can also affect multiple sexual signals (Martín *et al.*, 2008).

A good way to investigate sex differences related to parasite loads and coloration is to study species for which the males and females show the same trait (Almaa *et al.*, 2018). Host-parasite relations often differ between sexes because of differences in host traits (such as hormone levels, morphology or immune system) which can lead to the adaptations of the parasite to one sex, and in return adaptations related to the expression of the infection that are sex-specific to the host (Duneau and Ebert, 2012). In fact, these differential host-parasite relationships can have a dissimilar effect on color expression between males and females even in a species where both sexes show the same ornament, as shown for *Gallotia* lizards where only male's cheeks presenting high UV hue (associated with a high degree of infection) correlates with a higher body condition whereas females with whitish cheeks are in better condition (Megía-palma *et al.*, 2016). The yellow, orange and red colors shown by many reptiles are induced by carotenoids and pterins in the dermal cells (Steffen *et al.*, 2015). Pterins are rich in nitrogen and UV-fluorescent compounds (Moon *et al.*, 2010) whereas carotenoids are lipo-soluble pigments that animals store in fat once acquired from the diet because they cannot be synthesized by most animals (Steffen *et al.*, 2015). Carotenoids can also be used to remove reactive oxygen species from the system, which indicates a possible trade-off between the showy yellow color and the immune system (Burton and Ingold, 1984). Thus, only the highest-quality individuals could afford to show these bright colors.

However, it has been shown in the painted dragon lizards (*Ctenophorus pictus*) that there is not a well-defined link between carotenoid being used for both bright colorations and as antioxidant (Olsson *et al.*, 2008). It is then unlikely that carotenoids represent an additional antioxidant advantage. Turtles use UV channels when processing what they are seeing and their visual system is complex enough to admit a wide range of chromatic combinations (Rocha *et al.*, 2008). In addition, one can assume that some turtles, such as the Painted Terrapin (*Callagur borneonsis*), may have the ability to recognize some portions of the color spectrum since adult males change color during breeding (Gibbons and Greene, 2009). Ketocarotenoids are carotenoids that contain a ketone group. These pigments are present in avian eyes, specifically in their long-wave sensitive cones (LWS) red oil droplets, but only recently a study has found that turtles also have them (Twyman *et al.*, 2016). Turtles are the only other tetrapod known to have these red oil droplets to date, which can be explained by the presence of the *CYP2J19* gene in their genome (Twyman *et al.*, 2016). Changes in this gene, that occurred later for some birds and turtle lineages, seem to link the apparition of red coloration and the discrimination between different shades of red (Twyman *et al.*, 2016). In addition, it is thought that these changes appeared convergently in both birds and turtles due to sexual selection (Prager and Anderson, 2010; Ibáñez *et al.*, 2014).

Because successful copulation amongst aquatic turtles usually depends on the receptivity of the females, males have to elaborate effective mating strategies (Berry and Shine, 1980; Ferrara *et al.*, 2009). Courtship behavior in freshwater turtles also differs according to sexual size dimorphism (SSD) (Liu *et al.*, 2013). For species in which the males are smaller than the females, males tend to show color markings as ornaments and signals instead of using aggressive courtship

behavior: males usually perform head bobbing and titillation instead of biting (Brejcha and Kleisner, 2016). Head bobbing may not only have a function in chemical signaling: it is also used to calm females and to display the coloration on the head (Liu *et al.*, 2013). As for titillation, males straighten their forelimbs and foreclaws before vibrating them quickly around the female's chin (Change *et al.*, 1933). That waving movement of the forelimbs near the female's neck and head presumably plays a role in calming the females, thus increasing reproductive fitness since female receptivity dictates successful copulation (Ferrara *et al.*, 2009). Furthermore, because males need to mount the females and curl their tail until both cloacae are touching to achieve effective copulation, having a longer tail can facilitate copulation for freshwater turtles (Gibbons and Greene, 2009).

I studied the Painted Turtle (*Chrysemys picta*), a species of freshwater turtle, to test if the color of their chin and the length of their claws, both putative secondary sexual characters, are affected by their endoparasite load, as predicted by the Hamilton and Zuk hypothesis. Female reproductive qualities have been shown to affect painted turtle males mate choices (Mc Guire *et al.*, 2014), it is thus evolutionary relevant for females to evolve and have epigamic traits as well as males. Although female turtles can sometimes use foreclaw display for social play (Liu *et al.*, 2013), they do not perform titillation as males do. Therefore, I predict that (1) individuals with fewer blood parasites should have more intense chin coloration and that (2) males with fewer blood parasites should have longer claws. Such results would help us identify clearly which traits can lead to better reproductive success amongst freshwater turtles. It could also clarify if there is an actual link between the degree of blood infection and the intensity of coloration to possibly add support to the Hamilton and Zuk hypothesis in turtles.

Methods:

Study site and species:

Painted turtles can be found either in the United States or in Southern Canada and they can live in almost any aquatic or semi-aquatic habitat such as wetlands, lakes, swamps, and rivers (Gibbons and Greene, 2009). Painted turtles are one of the most studied turtle species, probably because they are the most abundant. Their bright colors varying from olive green to black with red or yellow stripes and curved or straight red lines on the marginals scutes also seems to be of interest for many. Their heads are ornamented with yellow stripes with a yellow spot behind the eyes. On the anterior portion of the chin, one will usually find two yellow stripes that reconnect further down the chin (see Fig. 1). As for the posterior part of the chin, it varies from bright yellow to red. Courtship happens from March to mid-June when the temperature begins to increase whereas nesting happens from late May to mid-July.

From July to mid-August of 2018, I used hoop nets to capture turtles. They were verified every 24 hours and stayed installed at the same location for 4-5 days before being removed. Every week, I installed the hoop nets in new locations to collect data in a broader range, with a total of 37 locations (see Fig.2) along the Rideau Canal (Ontario, Canada): Clear lake (1 site and n = 9 painted turtles), Newboro lake (12 and n = 46), Sand lake (12 and n = 14) and Upper lake (12 and n = 19). After sorting turtles, a total of n = 59 (25 females and 34 males) were available for further analysis (some had amputated legs, non-usable photographs or blood sample could not be obtained for various reasons). Permits from Park Canada Agency (Research and

Collection #RIC-2018-29178) and Wildlife Scientific Collector's Authorization (#1089358) were obtained prior to capture.

Field measurements:

For every turtle caught, the length of the tail and the carapace were measured with a caliper and the weight noted. From the weight and the carapace length, I calculated a body condition index for each turtle by plotting the log of the mass against the log of the carapace length (see Fig. 3). From the linear regression obtained, I estimated the expected value of the mass. I could then calculate the residual (actual value minus the expected value). The residuals were used as the index of the body condition, which is useful when the plot shows a linear relationship because it becomes a reliable estimation of the stored fat, thus the energy reserves of the individual (Green, 2001; Labocha *et al.*, 2014). Pictures of the chin and the claws were taken against a grey plastic cardboard with a ruler as a reference scale. From the pictures of the claws, the average length was calculated with respect to the ruler showed on each picture using ImageJ (Schneider *et al.*, 2012). The number of leeches was also counted (ectoparasite load) since they are known to be the primary vector for blood parasites (Siddall and Dessler, 2001). I inspected every turtle's limbs, tail, carapace, plastron, neck, and head to find leeches. Sex was determined based on secondary sexual characteristics such as claw length and position of the cloaca. Before being released at its capture point, a blood sample from the caudal vein was taken in order to produce one blood smear per individual for further blood parasites analysis (see below) and a unique combination of mark on the marginal scutes was applied to clearly identify each turtle if recaptured.

Screening of blood smears for parasites:

Blood samples from the caudal vein were used to make one blood smear per turtle, which once dried was fixed in methanol for 1 minute. Blood smears were stained as soon as they were dry with Wright-Giemsa stain (Fisher Scientific Company, Middleton, Virginia, USA) following the Sigma-Aldrich protocol (Sigma-Aldrich, 2014). Before being placed into a slides storage box to dry, any leftover taint was removed by being rinsed with distilled water. Fixing the blood smear in methanol preserves the cell morphology while staining them with Wright-Giemsa makes any hemiparasites stand out by appearing as dark blue against the varying shades of pink of the cytoplasm from the red blood cells during compound microscopy analysis (Sigma-Aldrich, 2014) (see Fig. 4). I counted the number of parasites in the blood smears by observing 500 erythrocytes in each one of them with a compound microscope (Olympus CX41) at 1000x magnification with an oil-immersion lens to assess the degree of infection for each turtle (Bulté *et al.*, 2009).

Photographs:

All photographs were taken with a Nikon Coolpix p500 with a wide 36X Optical Zoom and a 12.1 megapixels resolution. Lighting conditions were variable since all pictures were taken outdoors, but because all photographs were linearized and equalized before any analysis, this was not an issue. Since this camera does not have RAW support, all files were saved in JPEG (Joint Photographic Experts Group). Even though many studies recommend using TIFF (Tagged Image File Format) or RAW files instead of JPEG (Stevens *et al.*, 2007), when the average RGB value is measured over several 100 pixels, the level of compression should not substantially or negatively impact the results (Bergman and Beehner, 2008). In fact, Bergmann and Beehner (2008) tested

this by comparing data obtained from paired JPEG and TIFF pictures (taken with less than 30 seconds interval) and found that the RGB values from all the JPEG and the TIFF files were within 5 % of each other.

Linearization and equalization of photographs:

Before using the pictures of the turtle's chin for further analysis, it was necessary to linearize and equalize them. That transformation of the data is important because otherwise a greyscale value of 100 is not in fact 2 times brighter than a greyscale value of 50 (Johnsen, 2016). The first step was to photograph gray standards of distinct reflectance from X-Rite ColorChecker passport in different lighting conditions. This step is called the calibration. Since cameras are not calibrated like a spectrophotometer, you will not obtain a clear measure of color intensity. Therefore, adding a grey standard in each photograph is the same as adding a scale reference. Once the pictures were taken, a central square of 100 x 100 pixels was cut out from each standard and their average RGB values were extracted with the ImageJ program (Schneider *et al.*, 2012). These average color scores could then be plotted against their known reflectance value (Q), which were 3.1 %, 9 %, 19.7 %, 36.2 %, 59.1 %, and 90 %. For every lighting condition (4), a set of 3 functions (one for each R – red, G – green, and B – blue sensor) was drawn (Johnsen, 2016). The linearization equations used were:

$$(1) Q_R = a_1 \times b_1^R$$

$$(2) Q_G = a_2 \times b_2^G$$

$$(3) Q_B = a_3 \times b_3^B$$

These linearization equations are also called the calibration curves since they are the same for each channel, with only the RGB pixel value varying whereas a and b are specific constants for each camera sensor. The *nls* function in R (Version 3.5.2 GUI 1.70 El Capitan Build) was used to estimate the constant a and b for equations 1-3. After the linearization, the next step was to confirm that each camera sensor gives equal Q value for the same grey standard. Equalization was therefore confirmed by paired t-tests, meaning that the linearization also equalized the channels in this case (Paterson and Blouin-Demers, 2017). Using equations 1-3, the third step was to linearize and equalize all pictures of turtles with respect to the grey standard (18 % reflectance) in each picture.

Color intensity quantification:

Most cameras have non-linear output related to light intensity and for the RGB values to be used and accurately identify the color intensity of the targeted object, all the transformations explained above are needed before performing color intensity quantification (Stevens *et al.*, 2007). All the linearized images should also be put on the same scale (Teasdale *et al.*, 2013). I chose to rescale them to 180 pixels per inch with Photoshop Adobe Software because it is the measurement that fitted best all photographs. I then placed all the photographs on a white background. No pixels are added or removed when the resolution is changed. It only changes the number of pixels per inch (PPI). The color intensity of the chin of every turtle was then quantified by measuring the average RGB values with ImageJ (Schneider *et al.*, 2012). These values were then linearized with respect to equation 1-3 (with constant a and b depending on the light condition) in order to compare the variation of production of color between individuals.

Statistical analysis:

First, I used a principal component analysis (PCA) with 3 variables (the R, G and B average values for each individual) to determine if variation in the three colors can be summarized. Secondly, I analyzed factors affecting the color intensity with a general linear model (GLM), using a single response variable combining R, G and B derived from the result of the PCA. In my model, I included the sex, the degree of infection (number of blood parasites), the carapace length and the body condition as predictor variables. I included sex since previous work on the effect of blood parasites on their host have shown sex-specific differences (Folstad and Karter, 1992; Martin *et al.*, 2007b; Duneau and Ebert, 2012). I included the carapace length because it is an indicator of a size and (1) larger individuals tend to have greater fitness (“bigger is better” hypothesis) (Janzen, 1993) and (2) because larger turtles have a greater skin surface exposed to leeches, and thus a possible higher probability of being infected. I included a body condition index because there is evidence in the literature that individuals with lower body condition are more susceptible to parasites since they usually invest fewer resources in their immune system (Smallridge and Bull, 2000; Navarro *et al.*, 2003). I also analyzed factors affecting the color intensity with a multivariate analysis regression with three response variables (R, G and B) instead of the single derivate response variable used in my first GLM. The predictor variables were the same as the one in the first GLM (sex, the degree of infection, carapace length and body condition). Finally, I analyzed factors affecting the average claw length (response variable) by replicating my first GLM with the removal of the sex predictor. Since it is only relevant for males to have longer claws, I excluded the females for this analysis.

Results:

Body measurement and parasite count:

The prevalence of hemiparasite in the population is 86.4 % (51 / 59) with an average of 5.31 ± 3.98 parasites (maximum of 17 hemiparasites) for every 500 erythrocytes counted, whereas only 37.3 % (22 / 59) of the turtles had leeches as ectoparasites on them at the moment of capture. There was no significant difference in the average number of blood parasite between male and female ($t_{(49)} = 0.235$, $p = 0.815$). Females were significantly larger than males with respect to plastron length and mass ($t_{(37)} = 1.886$, $p < 0.01$; $t_{(35)} = 2.691$, $p < 0.05$) but females did not have significantly larger carapace ($t_{(37)} = 1.886$, $p = 0.06$). Males had in average longer tail and longer claws than females ($t_{(42)} = 2.743$, $p < 0.01$; $t_{(57)} = 7.263$, $p < 0.01$).

Linearization and Equalization of photographs:

Different constant a and b seemed to linearize the RGB value depending on the light condition. In sunny condition, constants a and b found by *nls* function in R were: $a_1 = 1.8985$, $b_1 = 1.016$, $a_2 = 1.7835$, $b_2 = 1.016$, $a_3 = 1.545$, $b_3 = 1.017$. In cloudy condition, constants a and b found by *nls* function in R were: $a_1 = 3.7815$, $b_1 = 1.015$, $a_2 = 3.642$, $b_2 = 1.015$, $a_3 = 3.606$, $b_3 = 1.016$. For the sunny (2) and cloudy (2) light conditions, paired t-tests were performed to confirm equalization. Sunny light condition 1 ($t_{(5)} = 1.975$, $p = 0.106$ for RG, $t_{(5)} = 0.680$, $p = 0.527$ for GB and $t_{(5)} = 1.696$, $p = 0.151$ for BR) and sunny light condition 2 ($t_{(5)} = 2.112$, $p = 0.088$ for RG, $t_{(5)} = 1.941$, $p = 0.110$ for GB and $t_{(5)} = 1.696$, $p = 0.151$ for BR) had equalized data after linearization. Cloudy light condition 1 ($t_{(5)} = 2.148$, $p = 0.084$ for RG, $t_{(5)} = 0.643$, $p = 0.549$ for GB and $t_{(5)} = -2.281$, $p = 0.071$ for BR) and cloudy light condition 2 ($t_{(5)} = 1.375$, $p = 0.228$ for RG, $t_{(5)} = 2.373$, $p = 0.064$ for GB and $t_{(5)} = 1.516$, $p = 0.191$ for BR)

also had equalized data after linearization. The number of iterations to convergence was always smaller than 10. As for the achieved convergence tolerance, they were all lower than 1×10^{-5} .

Color intensity quantification:

For turtles photographed in sunny conditions ($n = 21$), the linearized RGB averaged values ranged from 5.45 to 41.74 with an average of 11.45 ± 8.55 for the red channel, 5.67 to 13.64 with an average of 7.67 ± 2.09 for the green channel and 3.56 to 10.45 with an average of 5.34 ± 1.76 for the blue channel. As for the turtles photographed in cloudy conditions ($n = 38$), the linearized RGB averaged values ranged from 8.04 to 41.73 with an average of 18.46 ± 7.38 for the red channel, 8.00 to 15.24 with an average of 11.83 ± 2.37 for the green channel and 7.29 to 13.36 with an average of 9.24 ± 1.48 for the blue channel.

Identifying predictors of color intensity and claw length:

The first principal component (PC1) from the PCA explained 77.9% of the variance in chin coloration, whereas PC2 explained 18% and PC3 was negligible. Since the loading values from R, G and B were similar (see Table 1), PC1 was used to derive one single value of chin color intensity for every individual. That value was the response variable from the first general linear model. None of the predictor variables were significant (see Table 2). Results from the multivariate analysis are summarized in Table 3. Apart from the degree of infection being on the edge of significance ($t_{(54)} = -2.015$, $p = 0.0489$) for the red proportion of color intensity in the turtle's chin, no other predictors were significant for either R, G or B. Finally, none of the predictor variables were significant predictor of the average claw length for males (see Table 4).

Discussion:

Body measurement supports the use of secondary sexual characters as identifiers of sex:

Sex seems to have an impact on body size. Measures of the plastron length and the mass coincide with the female-biased sexual size dimorphism (FBSSD) present in the Painted turtle species. FBSSD is common in a wide range of taxa [arthropods, reptiles, fish and even some birds (Webb and Freckleton, 2007)] and the primary purpose of this difference is often thought to be that females are under selection for fecundity associated with a large abdominal cavity whereas males are under selection for mobility (Bonnet *et al.*, 2010). Interestingly, the carapace length did not support the expected FBSSD. However, even if the straight carapace length measurement is considered the standard body size measurement in turtles, it seems that mass has potentially a greater statistical power with respect to the detection of SSD (Regis and Meik, 2017). Further research should consider a curvilinear measurement of the shell that takes into account both height and width (shell shape rather than shell length) to obtain a more precise/inclusive body size index (Gibbons and Lovich, 1990; Bonnet *et al.*, 2010).

Discrepancy between endo and ectoparasite prevalence:

The prevalence of blood parasites (86.4 %) was surprisingly higher than the prevalence of ectoparasites (37.3 %). Leeches are considered primary hosts and vectors of blood parasites in aquatic turtles (Siddall and Dessler, 1990, 1991) and so I expected similar prevalence for both endo and ectoparasites. Because leeches can have many different hosts in their lifetime, the number of leeches attached to the turtle at the time of capture may not accurately reflect the total number of leeches encountered during that turtle's whole life. Alternatively, basking species may have higher endoparasite load based on the fact that the multiplication rate of blood parasites often increases

as a function of temperature (Oppliger *et al.*, 2009). For instance, higher body temperatures were associated with higher blood parasite load in the common lizard (*Lacerta vivipara*) (Oppliger *et al.*, 2009). Panek (2006) did not identify this relationship in a study involving the Painted turtle but found a significant negative correlation between basking time budget and the number of leeches attached to females. This relation supports Cagle's (1950) "desiccating leech" hypothesis, and so the basking behavior may reduce the external parasite load by drying out the leeches (Panek, 2006). I have some reservations concerning the "desiccating leech" hypothesis since there seems to be a lack of support for this hypothesis (Ryan and Lambert, 2006; McCoy *et al.*, 2007) and because leeches can support a tremendously high level of desiccation [up to 92% loss of the water contained in their body (Hall, 1922)]. Nevertheless, there are many cases among other reptiles where the ingestion of infected arthropods led to the transmission of Haemogregarines (Smith, 1996). Hemogregarines (Apicomplexa: Adeleorina) are known for their ability to cross-infect many different hosts such as snakes, frogs, lizards, and turtles (Siddall and Desser, 2001), and so are often considered as common reptile's hemoparasites (Rossow *et al.*, 2013). For instance, Brown *et al.* (2006) demonstrated that young keelback snakes (*Tropidonophis mairii*) ingesting infected frogs become infected with haemogregarines. Therefore, leech's attachment may not be mandatory for a turtle to get infected with haemogregarines since they can penetrate the gut wall of a wide range of vertebrates (Smith, 1996).

Male's average claw length seems to not be affected by endoparasite load:

The endoparasite load seems to have no effect on the painted turtle male's average claw length. My results support the presence of sexual dimorphism in the Painted turtle species and suggest that the male's elongated claws are a secondary sexual character. I assume that the

significantly longer tail and claws in male's Painted turtle can be considered as secondary sexual characters because it has been previously shown that the tail and claws growth in male's Slider turtle (*Pseudemys scripta troostii*) occur during sexual maturation and are likely to be under the control of testosterone (Evans, 1951). Although I did not quantify the effect of the claw length on fitness, the literature shows that this elongation of the claws is in fact considered a secondary sexual character for many aquatic turtle's species associated with FBSSD (Hidalgo, 1982; Liu *et al.*, 2013; Brejcha and Kleisner, 2016). The difference in the average claw length was significant between males and females, but the effect of blood parasites on male's claw length may have been overseen because all claws were considered. During courtship, males are mostly using the second and the third claws to perform titillation and so these claws are usually the elongated ones (Murphy and Lamoreaux, 1978). Therefore, the average length of all 5 claws may not be a representative measure of this secondary sexual character. Also, it is well known that small samples do not provide a precise estimate of the strength of the relationship between the response variable and the predictors. It is thus possible that a relation between the claw length and the endoparasite do exist, but my design was not strong enough to detect it ($n = 34$). Because the elongation of the third claw can be used as an indicator of males painted turtle attainment of sexual maturation (Cagle, 1948; Frazer *et al.*, 1993), the sole use of that third claw length in my model would have been a more suitable approach to detect any effect of blood parasites on that trait. Therefore, if I was not constrained by time, I would definitely run my model again with only the third claw length as the response variable.

Color intensity seems to not be affected by endoparasite load:

This study did not find support for the Hamilton and Zuk hypothesis in the Painted turtle. When the average R, G, and B values were considered separately in the multivariate analysis, the proportion of red was the only color for which the degree of infection was a significant predictor for the color intensity. Because the p-value was on the edge of significance ($p = 0.0489$) and the effect size was small, it is unlikely that the intensity of color in the Painted turtle's chin is associated with the endoparasite load.

The lack of support for the Hamilton and Zuk hypothesis in this study may be due to my experimental design. The results may have been altered because of the loss of too many details since every photograph were saved under JPEG files. Photographs saved under JPEG format get a varying level of compression of the color and the spatial information, resulting in the loss of information that cannot be retrieved (Stevens *et al.*, 2007). Thus, further study should save all photographs under TIFF or RAW format. Instead of using digital photography, spectrophotometry could have been used given how precise the measures it gives can be (Bergman and Beehner, 2008). However, spectrophotometry can only sample a very small area at a time (point samples) (Stevens *et al.*, 2007). It is possible to take many measurements of reflectance within a specific area with a spectrophotometer and then calculate an average measure (see Bulté *et al.*, 2013) but given the large size of the area I wanted to examine, the choice of the exact location to analyze would be problematic. Using digital photography was thus a suitable and fast alternative that allowed color variation quantification by gathering a great deal of data quickly with little equipment and with the use of ImageJ it was then possible to extirpate a large amount of data from my pictures (Stevens *et al.*, 2007). Unfortunately, a limitation to digital photography is its inability

to quantify the UV spectrum, making it impossible to assess how other animals that see in the UV spectrum are perceiving these color variations (Bergman and Beehner, 2008). A standard camera would not register a difference between blue bioluminescence and violet, it would only associate both as being blue color (Johnsen, 2016). Since turtles are known to see in the UV spectrum (Rocha *et al.*, 2008), and UV coloration can be used in some species as an indicator of an individual quality (Doucet and Montgomerie, 2003), this study may be considered as an initial step in order to quantify variation of color production before UV variations. This may explain in part why the predicted results were not obtained. Lastly, because pterins are UV-fluorescent compounds (Moon *et al.*, 2010), further study should consider the investigation of UV variation.

Even if major improvements can be done to increase the strength of this study, it is indispensable to consider the option that the bright colors of the Painted turtles may have no function in either sexual selection or signaling. For instance, a study investigating differences in the spectral reflectance of the carapace, plastron, yellow and red ornaments on the head and limbs between male and female's Painted turtle found no significant difference in color brightness (Rowe *et al.*, 2014). This study, therefore, suggests that there is no link between the coloration showed by the Painted turtle and sexual selection. It has also been shown in the Common Wall lizard that yellow-colored skin and orange/red colored skin are under the control of 2 small regions of the genome that differs in sequences and that changes in these sequences are prone to have pleiotropic effects (Andrade *et al.*, 2019). Therefore, the degree of expression of either carotenoid or pterin may still have effects on the fitness or other traits of an individual. In fact, genes regulating the production of pterins pigments also regulates other physiological processes and coloration could then be considered a by-product with no signal function (Reinke *et al.*, 2017; Andrade *et al.*, 2019).

As for carotenoids, it is well known that they provide many health benefits (cancer prevention, general immunity, wound healing, stabilization of proteins and membranes, etc.) (Olson and Owens, 1998) and that the Painted turtle's numerous yellow stripes are known to help with camouflage for turtles that live in a vegetated area (Gibbons and Greene, 2009). However, carotenoids can become pro-oxidants at high oxygen concentration, which can have a hazardous impact on the system (El-Agamey *et al.*, 2004). To prevent such damages, the excess pigments could be redirected externally (skin, plastron, carapace) (Reinke *et al.*, 2017), explaining why painted turtles express such bright colors throughout their whole life. Because they are also anoxia-tolerant species, it is even more evolutionary relevant for them to «store» these antioxidants that can help with recovery (Reinke *et al.*, 2017). Moreover, newly hatched turtles often have brighter colors than adult one and seem to only be differing in size with little to no difference with adults of that species (Gibbons and Greene, 2009). Knowing that males and females become sexually mature between 5-8 years (Mahmoud, 1967), their chin color intensity is most likely to decrease than increase by the time they get sexually mature. Finally, it is not because the endoparasite load did not affect the Painted turtle coloration that it does not have any effect on their fitness. Hemogregarines can be quite large in size and therefore shift their host cell nuclei and deform the shape of the blood cell (Rossow *et al.*, 2013), and deforming the shape of the cells can inhibit normal cell function (Smith, 1996). The endoparasite prevalence (86.4 %) and the highest degree of infection (3.4 %) were both high when compared to other studies [prevalence of 41% and intensity of infection rarely above 1 infected cell for every 10^4 blood cells in the Painted turtle species (Siddall and Desser, 2008), highest degree of infection of 2.2% in the Black River turtle (*Rhinoclemmys funerea*) and 0.08% in the White-Lipped mud turtles (*Kinosternon leucostomum*) (Rossow *et al.*, 2013)]. Thus, the potential impact of these high level of infection

on performance is not negligible and it could be interesting to investigate this as well in further research.

Conclusion:

In conclusion, my study does not support the Hamilton and Zuk hypothesis for the Painted turtle. The experimental design could use improvements, but I conclude that it is unlikely that the bright colors of the Painted turtle are sex-linked or used in signaling. I also cannot concretely conclude if the male's claw length is affected by endoparasite load. More research with stronger designs is needed to determine the effect of blood parasite on the Painted turtle physiology, development and appearance.

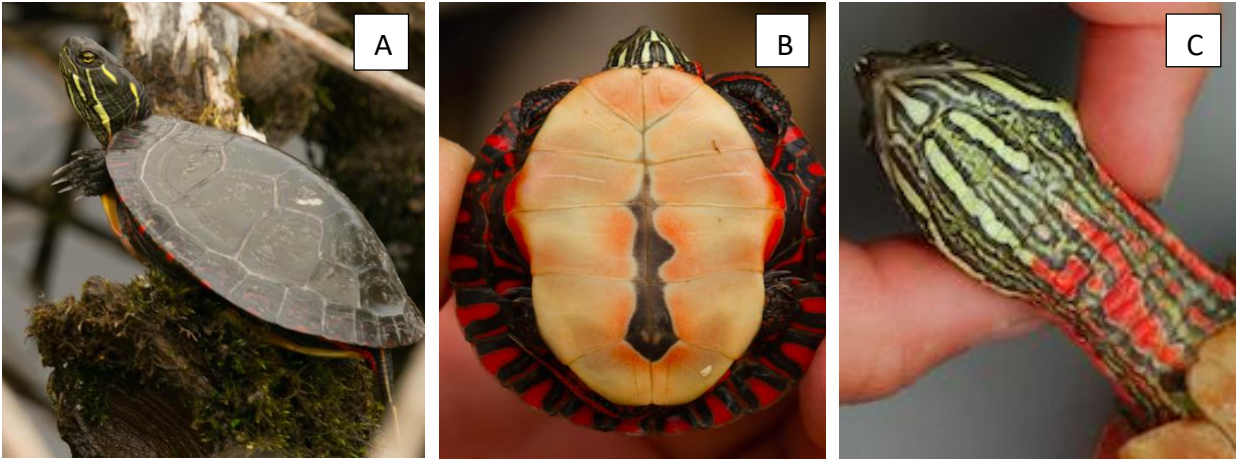


Figure 1. Pictures of Painted Turtles (*Chrysemys picta*) from Rideau Canal, Ontario, showing (A) yellow ornaments on the head, (B) red pattern on the marginal scutes and (C) yellow upper part of the chin and red lower part of the chin. Pictures A and B were obtained from Dr. Grégory Bulté (Carleton University, Department of Biology).

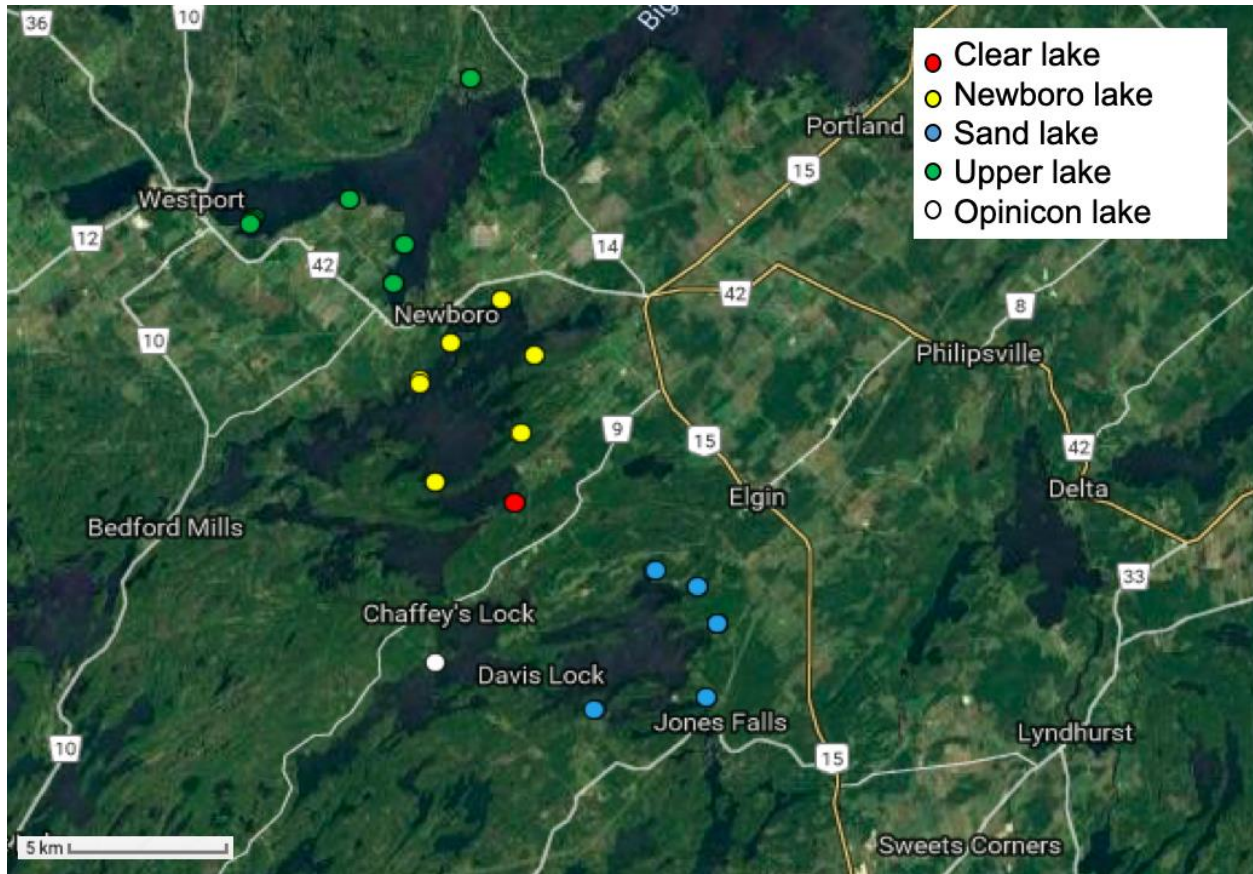


Figure 2. Map of the locations (n = 37) where the hoop nets were installed for capture along the Rideau Canal (Ontario, Canada) from July to mid-August of 2018.

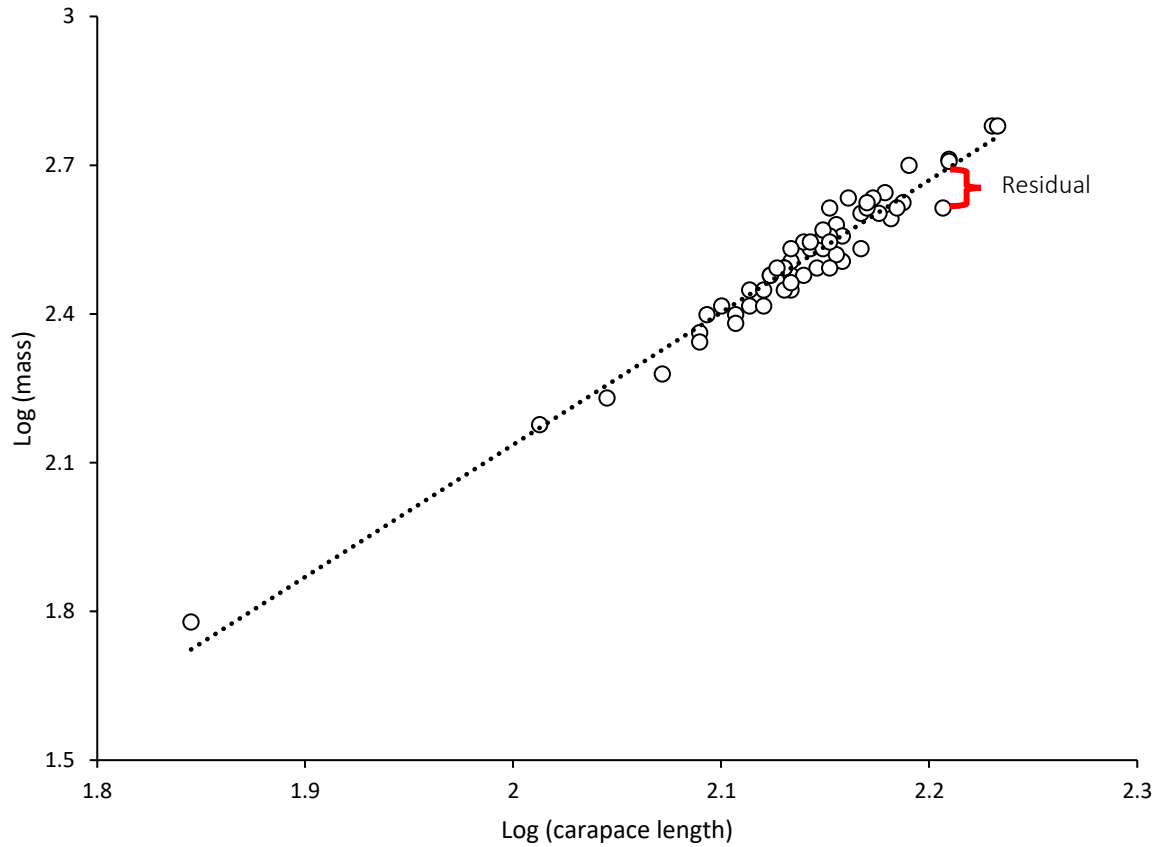


Figure 3. Log of the mass as a function of the log of the carapace length. The linear regression function is $y = 2.6668x - 3.1972$ and the correlation coefficient is $R^2 = 0.9545$. The residual is the difference between the actual mass value and the expected mass value based on the linear regression, which represents the body condition index and can be measured for each individual.

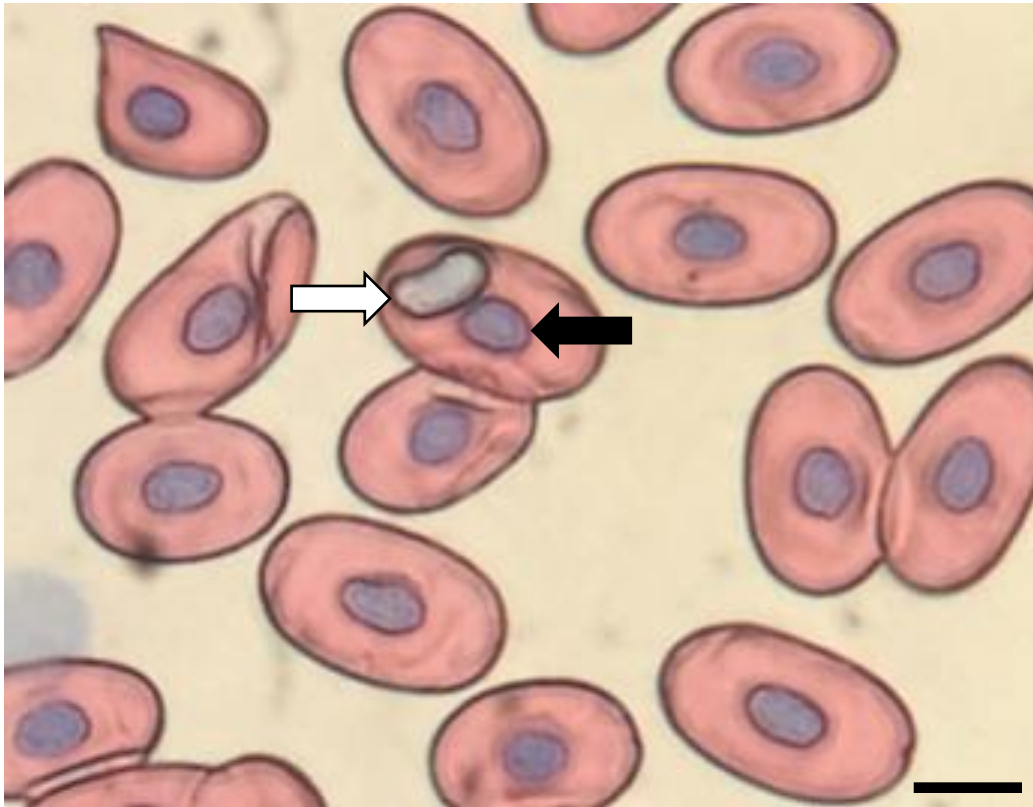


Figure 4. Endoparasite (white arrow) infecting a red blood cell (black arrow) of Painted turtles (*Chrysemys picta*) from Rideau Canal, Ontario, Canada (magnification is 1000x). Scale bar: 10 μ m.

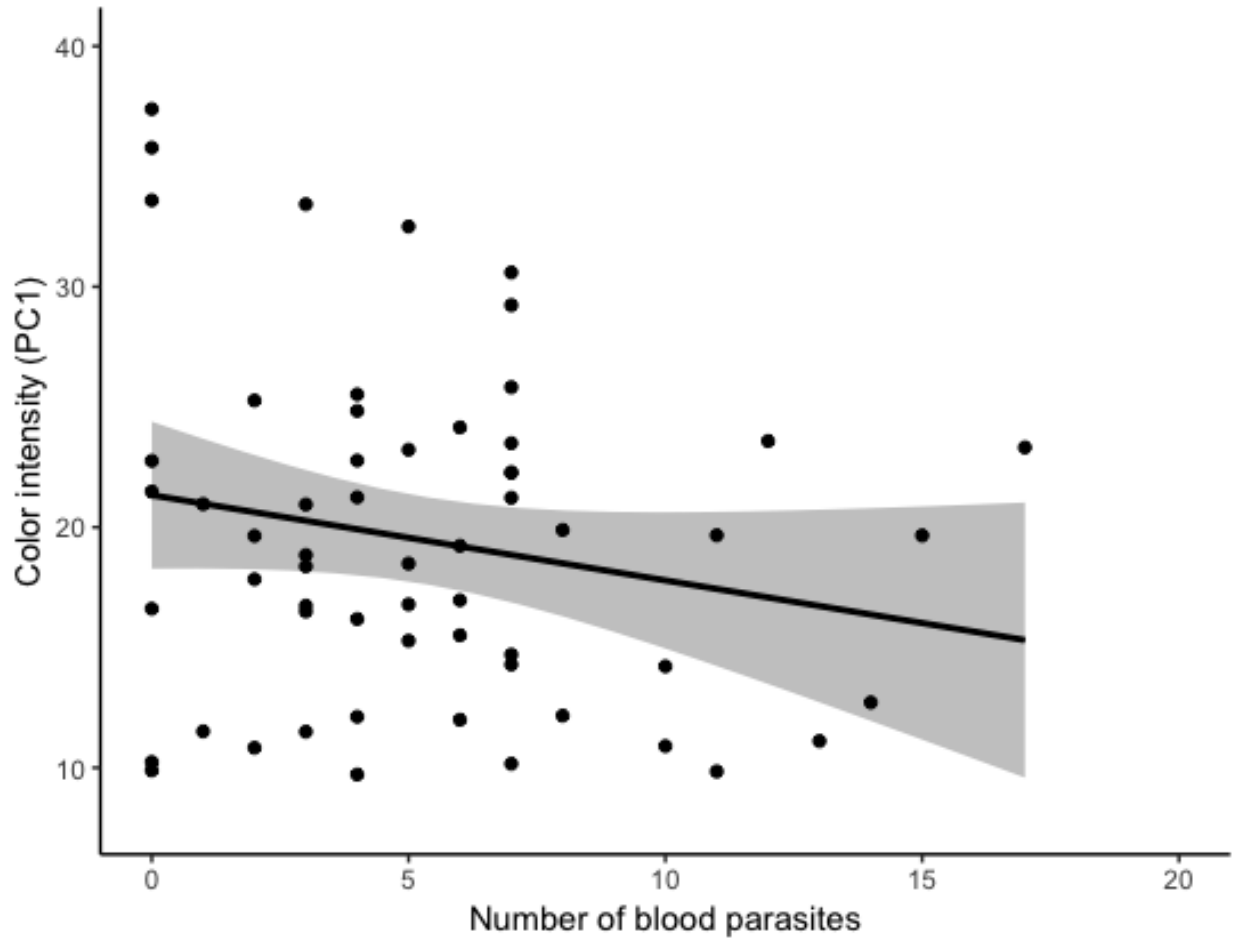


Figure 5. The relationship between the chin color intensity adjusted values of Painted turtles (*Chrysemys picta*) from Rideau Canal, Ontario, and the number of blood parasites. The regression line is black, and the grey shading displays the 95% confidence interval (n = 59).

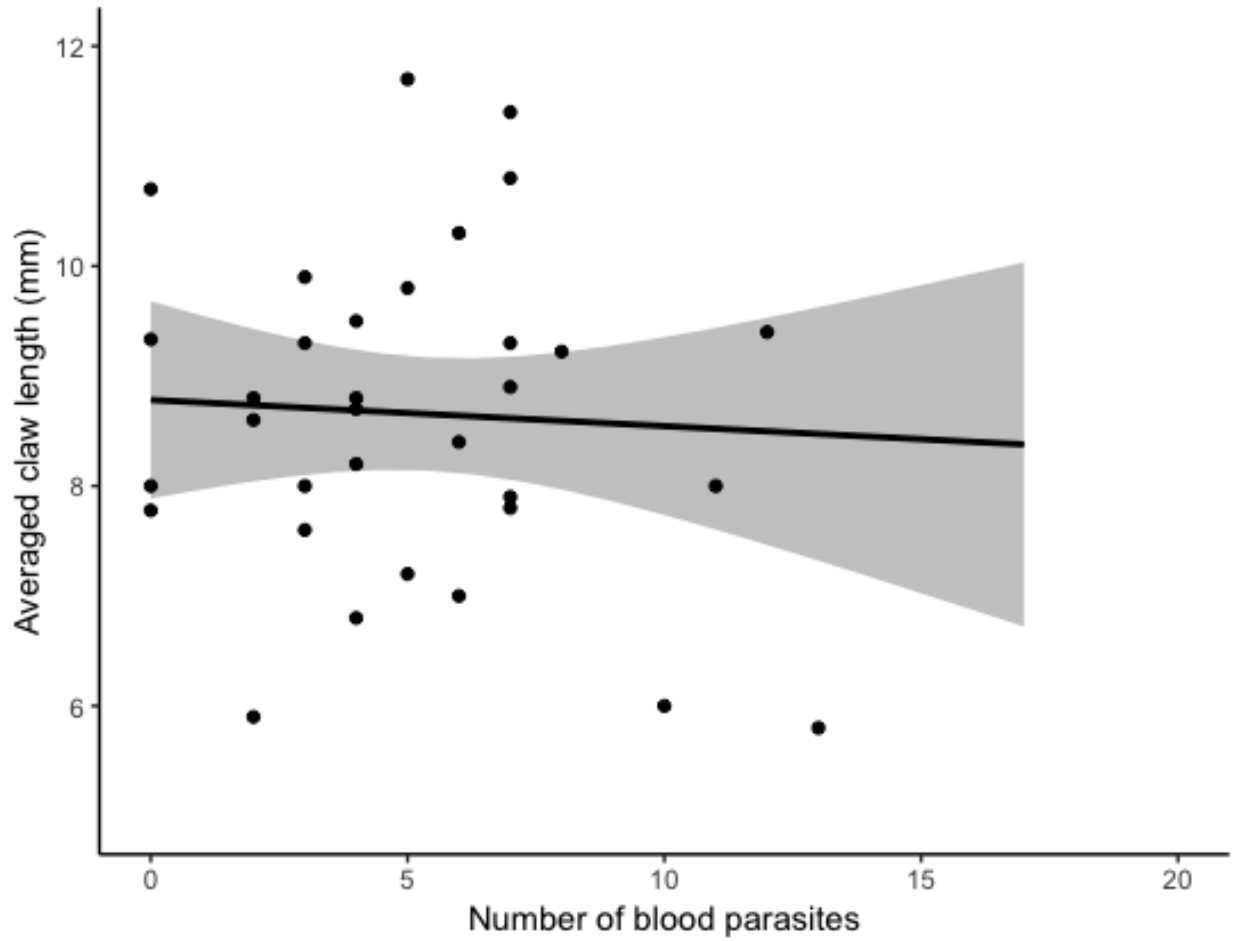


Figure 6. The relationship between the averaged claw length adjusted values (mm) of male's Painted turtles (*Chrysemys picta*) from Rideau Canal, Ontario, and the number of blood parasites. The regression line is black, and the grey shading displays the 95% confidence interval (n = 34).

Table 1. Variable loadings and summary for the three components from the principal component analysis quantifying the females (n = 25) and males (n = 34) Painted turtle's (*Chrysemys picta*) chin color intensity, based on the proportion of red (R), green (G) and blue (B) extracted with ImageJ from a digital photograph of each individual.

	PC1	PC2	PC3
R	0.5308052	0.76761967	0.3591738
G	0.6319421	-0.07610302	-0.7712701
B	0.5647079	-0.63637125	0.5254871
Standard deviation	1.5292	0.7455	0.32517
Proportion of variance	0.7795	0.1852	0.03525
Cumulative proportion	0.7795	0.9647	1.0000

Table 2. Model output for the first GLM analyzing the predictor variables (sex, the degree of infection, body condition and carapace length) for the chin color intensity of the Painted Turtle's (*Chrysemys picta*) males (n = 34) and females (n = 25). The response variable is one single value of chin color intensity derived from PC1 and combining R, G and B values. The adjusted $R^2 = 0.008$.

	Estimate	Standard error	t value	p-value
Intercept	13.92077	9.17303	1.518	0.135
Sex	-0.36279	2.62272	-0.138	0.890
Degree of infection	-0.35452	0.23133	-1.533	0.131
Body condition	-41.2219	38.8896	-1.060	0.294
Carapace length	0.05490	0.06217	0.883	0.381

Table 3. Model output for the multivariate analysis regression analyzing the predictor variables (sex, the degree of infection, body condition and carapace length) for the chin color intensity of the Painted Turtle's (*Chrysemys picta*) males (n = 34) and females (n = 25). For the R response variable, the adjusted $R^2 = 0.005$. For the G response variable, the adjusted $R^2 = 0.064$. For the B response variable, the adjusted $R^2 = 0.038$. The p-values with a level of significance <0.05 are indicated with a *.

	Estimate	Standard error	t value	p value
R				
Intercept	14.4415	11.23447	1.285	0.2041
Sex	0.04316	3.21212	0.013	0.9893
Degree of infection	-0.57089	0.28331	-2.015	0.0489 *
Body condition	-18.3028	47.6292	-0.384	0.7023
Carapace length	0.03249	0.07614	0.427	0.6713
G				
Intercept	6.10390	3.86822	1.578	0.1204
Sex	0.20223	1.10599	0.183	0.8556
Degree of infection	-0.06664	0.09755	-0.683	0.4974
Body condition	-28.3007	16.3996	-1.726	0.0901
Carapace length	0.03231	0.02622	1.232	0.2232
B				
Intercept	4.24615	3.19324	1.330	0.1892
Sex	-0.90933	0.91300	-0.996	0.3237
Degree of infection	-0.01660	0.08053	-0.206	0.8374
Body condition	-24.1227	13.5379	-1.782	0.0804
Carapace length	0.03053	0.02164	1.411	0.1641

Table 4. Model output for the second GLM analyzing the predictor variables (degree of infection, body condition, and carapace length) for the average claw length of the Painted Turtle's (*Chrysemys picta*) males (n = 34). The adjusted $R^2 = -0.019$. The p-values with a level of significance <0.05 are indicated with a *.

	Estimate	Standard error	t value	p-value
Intercept	6.85259	3.00569	2.280	0.0299 *
Degree of infection	-0.02380	0.06654	-0.358	0.7231
Body condition	-13.69712	10.1966	-1.343	0.1892
Carapace length	0.01235	0.02244	0.551	0.5960

Literature Cited:

- Almaa, R. M. E. G. I. A., Aranjeb, D. P., Eguerac, S. R., Nezd, J. M. A., Ooperb, R. D. C., Laimontb, P. B., Erinoa, S. M. and Inervob, B. S. (2018). Multiple color patches and parasites in *Sceloporus occidentalis* : differential relationships by sex and infection. *current zoology* 64, 703–711. doi: 10.1093/cz/zoy007.
- Andrade, P., Pinho, C., Pérez i de Lanuza, G., Afonso, S., Brejcha, J., Rubin, C.-J., Wallerman, O., Pereira, P., Sabatino, S. J., Bellati, A., Pellitteri-Rosa, D., Bosakova, Z., Bunikis, I., Carretero, M. A., Feiner, N., Marsik, P., Paupério, F., Salvi, D., Soler, L., While, G. M., Uller, T., Font, E., Andersson, L. and Carneiro, M. (2019). Regulatory changes in pterin and carotenoid genes underlie balanced color polymorphisms in the wall lizard. *Proceedings of the National Academy of Sciences* 116, 5633–5642. doi: 10.1073/pnas.1820320116.
- Bergman, T. J. and Beehner, J. C. (2008). A simple method for measuring colour in wild animals: Validation and use on chest patch colour in geladas (*Theropithecus gelada*). *Biological Journal of the Linnean Society* 94, 231–240. doi: 10.1111/j.1095-8312.2008.00981.x.
- Berry, J. F. and Shine, R. (1980). Sexual Size Dimorphism and Sexual Selection in Turtles (Order Testudines). *Oecologia* 44, 185–191.
- Bonnet, X., Delmas, V., El-Mouden, H., Slimani, T., Sterijovski, B. and Kuchling, G. (2010). Is sexual body shape dimorphism consistent in aquatic and terrestrial chelonians? *Zoology* 113, 213–220. doi: 10.1016/j.zool.2010.03.001.
- Brejcha, J. and Kleisner, K. (2016). Turtles Are Not Just Walking Stones: Conspicuous Coloration and Sexual Selection in Freshwater Turtles. *Biosemiotics* 9, 247–266. doi: 10.1007/s12304-015-9249-9.
- Brown, G. P., Shilton, C. M. and Shine, R. (2006). Do parasites matter? Assessing the fitness consequences of haemogregarine infection in snakes. *Canadian Journal of Zoology* 84, 668–676. doi: 10.1139/z06-044.
- Bulté, G., Plummer, A. C., Thibaudeau, A. and Blouin-Demers, G. (2009). Infection of Yarrow's Spiny Lizards (*Sceloporus jarrovii*) by Chiggers and Malaria in the Chiricahua Mountains, Arizona. *The Southwestern Naturalist* 54, 204–207. doi: 10.1894/PAS-07.1.
- Bulté, G., Germain, R. R., O'Connor, C. M. and Blouin-Demers, G. (2013). Sexual Dichromatism in the Northern Map Turtle, *Graptemys geographica* . *Chelonian*

- Conservation and Biology* 12, 187–192. doi: 10.2744/ccb-0995a.1.
- Burton, G. W. and Ingold, K. U. (1984). Beta-Carotene : An Unusual Type of Lipid Antioxidant. *American Association for the Advancement of Science* 224, 569–573.
- Cagle, R. F. (1948). Sexual Maturity in the Male Turtle , *Pseudemys scripta troostii*. *Copeia* 1948, 108–111.
- Cagle, R. F. (1950). The Life History of the Slider Turtle , *Pseudemys scripta troostii*. *Ecological Monographs* 20, 31–54.
- Change, G., Search, E. and Barbara, S. (1933). Observations on the courtship of Turtles. *The University of Kansas Science Bulletin* 21, 269–271.
- Chen, I., Stuart-fox, D., Hugall, A. F. and Symonds, M. R. E. (2012). Sexual selection and the evolution of complex color patterns in dragon lizards. *Evolution* 66, 3605–3614. doi: 10.1111/j.1558-5646.2012.01698.x.
- Doucet, M. and Montgomerie, R. (2003). Multiple sexual ornaments in satin bowerbirds : ultraviolet plumage and bowers signal different aspects of male quality. *Behavioral Ecology* 14, 503–509.
- Duneau, D. and Ebert, D. (2012). Host Sexual Dimorphism and Parasite Adaptation. *PLOS Biology* 10, 1–9. doi: 10.1371/journal.pbio.1001271.
- El-Agamey, A., Lowe, G. M., McGarvey, D. J., Mortensen, A., Phillip, D. M., Truscott, T. G. and Young, A. J. (2004). Carotenoid radical chemistry and antioxidant/pro-oxidant properties. *Archives of Biochemistry and Biophysics* 430, 37–48. doi: 10.1016/j.abb.2004.03.007.
- Evans, L. T. (1951). Effects of Male Hormone upon the Tail of the Slider Turtle , *Pseudemys scripta troostii*. *Science* 114, 277–279.
- Ferrara, C. R., Schneider, L., Vogt, R. C. and Burger, J. (2009). The role of receptivity in the courtship behavior of *Podocnemis erythrocephala* in captivity. *Acta Ethologica* 12, 121–125. doi: 10.1007/s10211-009-0062-9.
- Folstad, I. and Karter, A. J. (1992). Parasites, Bright Males, and the Immunocompetence Handicap. *The University of Chicago Press for The American Society of Naturalists* 139, 603–622.
- Frazer, N. B., Greene, J. L. and Gibbons, J. W. (1993). Temporal Variation in Growth Rate and Age at Maturity of Male Painted Turtles , *Chrysemys picta*. *The American Midland*

- Naturalist* 130, 314–324.
- Freeman, C. R. and Conor, G. (2018). Age and infection history are revealed by different ornaments in a warbler. *Oecologia* 188, 1025–1036. doi: 10.1007/s00442-018-4268-2.
- Gibbons, W. and Greene, J. (2009). *Turtles: The Animal Answer Guide*. The John Hopkins University Press Baltimore doi: 10.2307/41416582.
- Gibbons, J. W. and Lovich, J. E. (1990). Sexual Dimorphism in Turtles with Emphasis on the Slider Turtle (*Trachemys scripta*). *Hepretological Monographs* 4, 1–29.
- Green, A. J. (2001). Mass /Length Residuals : Measures of Body Condition or Generators of Spurious Results? *Ecology* 82, 1473–1483.
- Hall, F. G. (1922). The Vital Limit of Exsiccation of Certain Animals. *The Biological Bulletin* 42, 31–51. doi: 10.2307/1536720.
- Hamilton, W. J. and Poulin, R. (1997). The Hamilton and Zuk Hypothesis Revisited : A Meta-Analytical Approach. *Behaviour* 134, 299–320.
- Hamilton, W. D. and Zuk, M. (1982). Heritable True Fitness and Bright Birds : A Role for Parasites? *Science* 218, 384–387.
- Hidalgo, H. (1982). Courtship and Mating Behavior in *Rhinoclemmys pulcherrima incisa* (Testudines : Emydidae : Batagurinae). *Transaction of the Kansas Academy of Science* 85, 82–95.
- Hosken, D. J. and House, C. M. (2011). Sexual selection. *Current Biology* 21, R62–R65. doi: 10.1016/j.cub.2010.11.053.
- Ibáñez, A., Polo-cavia, N. and López, P. (2014). Honest sexual signaling in turtles : experimental evidence of a trade-off between immune response and coloration in red-eared sliders *Trachemys scripta elegans*. *Naturewissenschaften* 101, 803–811. doi: 10.1007/s00114-014-1219-6.
- Janzen, F. J. (1993). An Experimental Analysis of Natural Selection on Body Size of Hatchling Turtles. *Ecological Society of America* 74, 332–341.
- Johnsen, S. (2016). How to measure color using spectrometers and calibrated photographs. *Journal of Experimental Biology* 219, 772–778. doi: 10.1242/jeb.124008.
- Labocha, M. K., Schutz, H. and Hayes, J. P. (2014). Which body condition index is best? *Oikos* 123, 111–119. doi: 10.1111/j.1600-0706.2013.00755.x.
- Liu, Y., Davy, C. M., Shi, H.-T. and Murphy, R. W. (2013). Sex in the Half-Shell: A Review of

- the Functions and Evolution of Courtship Behavior in Freshwater Turtles. *Chelonian Conservation and Biology* 12, 84–100. doi: 10.2744/CCB-1037.1.
- Mahmoud, A. I. Y. (1967). Courtship Behavior and Sexual Maturity in Four Species of Kinosternid Turtles. *American Society of Ichthyologists and Herpetologists* 1967, 314–319.
- Martin, C. H. and Johnsen, S. (2007a). A field test of the Hamilton-Zuk hypothesis in the Trinidadian guppy (*Poecilia reticulata*). *Behavioral Ecology and Sociobiology* 61, 1897–1909. doi: 10.1007/s00265-007-0430-2.
- Martin, J., Puertac, M., Salvador, A., Veiga, J. P., Abelenda, M. and Lopez, P. (2007b). The cost of producing a sexual signal: testosterone increases the susceptibility of male lizards to ectoparasitic infestation. *Behavioral Ecology* 7, 145–150. doi: 10.1093/beheco/7.2.145.
- Martín, J., Amo, L. and López, P. (2008). Parasites and health affect multiple sexual signals in male common wall lizards, *Podarcis muralis*. *Naturewissenschaften* 95, 293–300. doi: 10.1007/s00114-007-0328-x.
- Mc Guire, J. M., Congdon, J. D., Scribner, K. T. and Nagle, R. D. (2014). Female reproductive qualities affect male painted turtle (*Chrysemys picta marginata*) reproductive success. *Behavioral Ecology and Sociobiology* 68, 1589–1602. doi: 10.1007/s00265-014-1768-x.
- McCoy, J. C., Failey, E. L., Price, S. J. and Dorcas, M. E. (2007). An Assessment of Leech Parasitism on Semi-aquatic Turtles in the Western Piedmont of North Carolina. *Southeastern Naturalist* 6, 191–202. doi: 10.1656/1528-7092(2007)6[191:aaolpo]2.0.co;2.
- Megía-palma, R., Martínez, J. and Merino, S. (2016). A structural colour ornament correlates positively with parasite load and body condition in an insular lizard species. *The Science of Nature* 103, 1–10. doi: 10.1007/s00114-016-1378-8.
- Møller, A. P. (1990). Effects of a Haematophagous Mite on the Barn Swallow (*Hirundo rustica*): A Test of the Hamilton and Zuk Hypothesis. *Evolution* 44, 771–784.
- Moon, Y., Kim, S., Park, Y. M. and Chung, Y. (2010). Sensing UV/blue: Pterin as a UV-A absorbing chromophore of cryptochrome. *Plant signaling & Behavior* 5, 1127–1130. doi: 10.4161/psb.5.9.12567.
- Murphy, J. B. and Lamoreaux, W. E. (1978). Mating Behavior in Three Australian Chelid Turtles (Testudines : Pleurodira : Chelidae). *Herpetologica* 34, 398–405.
- Navarro, C., Marzal, A., de Lope, F. and Møller, A. P. (2003). Dynamics of an Immune Response in House Sparrows *Passer domesticus* in Relation to Time of Day, Body

- Condition and Blood Parasite Infection. *Oikos* 101, 291–298.
- Olson, V. A. and Owens, I. P. F. (1998). Costly sexual signals: Are carotenoids rare, risky or required? *Trends in Ecology and Evolution* 13, 510–514. doi: 10.1016/S0169-5347(98)01484-0.
- Olsson, M., Wilson, M., Isaksson, C., Uller, T. and Mott, B. (2008). Carotenoid intake does not mediate a relationship between reactive oxygen species and bright colouration : experimental test in a lizard. *The Journal of Experimental Biology* 211, 1257–1261. doi: 10.1242/jeb.015065.
- Oppliger, A., Célérier, M. L. and Clobert, J. (2009). Physiological and behaviour changes in common lizards parasitized by haemogregarines. *Parasitology* 113, 433. doi: 10.1017/s003118200008149x.
- Panek, M. J. (2006). The relationship between basking behavior, parasite load, and growth rates in midland painted turtles. *Master's Thesis*.
- Paterson, J. E. and Blouin-Demers, G. (2017). Distinguishing discrete polymorphism from continuous variation in throat colour of tree lizards, *Urosaurus ornatus*. *Biological Journal of the Linnean Society* 121, 72–81. doi: 10.1093/biolinnean/blw024.
- Price, T. D. (1998). Sexual selection and natural selection in bird speciation. *Philosophical Transactions of the Royal Society B: Biological Sciences* 353, 251–260. doi: 10.1098/rstb.1998.0207.
- Read, A. F. (1987). Comparative evidence supports the Hamilton and Zuk hypothesis on parasites and sexual selection. *Nature* 328, 68–70. doi: 10.1038/328068a0.
- Regis, K. W. and Meik, J. M. (2017). Allometry of sexual size dimorphism in turtles: a comparison of mass and length data. *PeerJ* 5, 1–21. doi: 10.7717/peerj.2914.
- Reinke, B. A., Calsbeek, R. and Stuart-Fox, D. (2017). A test of an antipredatory function of conspicuous plastron coloration in hatchling turtles. *Evolutionary Ecology* 31, 463–476. doi: 10.1007/s10682-017-9892-5.
- Rocha, F. A. F., Saito, C. A., Silveira, L. C. L., De Souza, J. M. and Ventura, D. F. (2008). Twelve chromatically opponent ganglion cell types in turtle retina. *Visual Neuroscience* 25, 307–315. doi: 10.1017/s0952523808080516.
- Rossow, J. A., Hernandez, S. M., Sumner, S. M., Altman, B. R., Crider, C. G., Gammage, M. B., Segal, K. M. and Yabsley, M. J. (2013). Haemogregarine infections of three species of

- aquatic freshwater turtles from two sites in Costa Rica. *International Journal for Parasitology: Parasites and Wildlife* 2, 131–135. doi: 10.1016/j.ijppaw.2013.02.003.
- Rowe, J. W., Bunce, C. F. and Clark, D. L. (2014). Spectral reflectance and substrate color-induced melanization in immature and adult Midland painted turtles (*Chrysemys picta marginata*). *Amphibia-Reptilia* 35, 149–159. doi: 10.1163/15685381-00002934.
- Ryan, J. T. and Lambert, A. (2006). Prevalence and colonization of *Placobdella* on two species of freshwater turtles (*Graptemys geographica* and *Sternotherus odoratus*). *Journal of Herpetology* 39, 284–287. doi: 10.1670/0022-1511(2005)039[0278:ahurth]2.0.co;2.
- Schneider, C. A., Rasband, W. S. and Eliceiri, K. W. (2012). NIH Image to ImageJ : 25 years of image analysis. *Nature Methods* 9, 671–675. doi: 10.1038/nmeth.2089.
- Siddall, M. E. and Desser, S. S. (1990). Gametogenesis and Sporogonic Development of *Haemogregarina balli* (Apicomplexa: Adeleina: Haemogregarinidae) in the Leech *Placobdella ornata*. *The Journal Of Parasitology* 37, 511–520.
- Siddall, M. E. and Desser, S. S. (1991). Merogonic Development of *Haemogregarina balli* (Apicomplexa : Adeleina : Haemogregarinidae) in the Leech *Placobdella ornata* (Glossiphoniidae), Its Transmission to a Chelonian Intermediate Host and Phylogenetic Implications. *The Journal of Parasitology* 77, 426–436.
- Siddall, M. E. and Desser, S. S. (2001). Transmission of *Haemogregarina balli* from painted turtles to snapping turtles through the leech *Placobdella ornata*. *The Journal Of Parasitology* 87, 1217–1218. doi: [http://dx.doi.org/10.1645/0022-3395\(2001\)087\[1217:TOHBFP\]2.0.CO;2](http://dx.doi.org/10.1645/0022-3395(2001)087[1217:TOHBFP]2.0.CO;2).
- Siddall, M. E. and Desser, S. S. (2008). Prevalence and intensity of *Haemogregarina balli* (Apicomplexa: Adeleina: Haemogregarinidae) in three turtle species from Ontario, with observations on intraerythrocytic development . *Canadian Journal of Zoology* 70, 123–128. doi: 10.1139/z92-018.
- Sigma-Aldrich (2014). *Wright-Giemsa Stain*.
- Smallridge, C. J. and Bull, C. M. (2000). Prevalence and intensity of the blood parasite *Hemolivia mariae* in a field population of the skink *Tiliqua rugosa*. *Parasitology Research* 86, 655–660. doi: 10.1007/PL00008547.
- Smith, T. G. (1996). The Genus *Hepatozoon* (Apicomplexa : Adeleina). *The Journal Of Parasitology* 82, 565–585.

- Prager, M. and Andersson, S. (2010). Convergent evolution of red carotenoid coloration in widowbirds and bishops (*Euplectes* spp.). *Society for the Study of Evolution* 64, 3609–3619.
- Steffen, J. E., Learn, K. M., Drumheller, J. S., Boback, S. M. and McGraw, K. J. (2015). Carotenoid Composition of Colorful Body Stripes and Patches in the Painted Turtle (*Chrysemys picta*) and Red-Eared Slider (*Trachemys scripta*). *Chelonian Conservation and Biology* 14, 56–63. doi: 10.2744/ccab-14-01-56-63.1.
- Stevens, M., Parraga, C. A., Cuthill, I. C., Partridge, J. C. and Troscianko, T. s. (2007). Using digital photography to study animal coloration. *Biological Journal of the Linnean Society* 90, 211–237. doi: doi:10.1111/j.1095-8312.2007.00725.x.
- Svensson, E. I., Mcadam, A. G. and Sinervo, B. (2009). Intralocus sexual conflict over immune defense, gender load, and sex-specific signaling in a natural lizard population. *The Society for the Study of Evolution* 62, 3124–3135. doi: 10.1111/j.1558-5646.2009.00782.x.
- Swierk, L. and Langkilde, T. (2013). Bearded ladies : females suffer fitness consequences when bearing male traits. *Biology Letters* 9, 1–4.
- Teasdale, L. C., Stevens, M. and Stuart-Fox, D. (2013). Discrete colour polymorphism in the tawny dragon lizard (*Ctenophorus decresii*) and differences in signal conspicuousness among morphs. *Journal of Evolutionary Biology* 26, 1035–1046. doi: 10.1111/jeb.12115.
- Twyman, H., Valenzuela, N., Literman, R., Andersson, S. and Mundy, N. I. (2016). Seeing red to being red: Conserved genetic mechanism for red cone oil droplets and co-option for red coloration in birds and turtles. *Proceedings of the Royal Society B: Biological Sciences* 283, 1–7. doi: 10.1098/rspb.2016.1208.
- Webb, T. J. and Freckleton, R. P. (2007). Only half right: Species with female-biased Sexual Size Dimorphism consistently break Rensch’s rule. *PLoS ONE* 2,. doi: 10.1371/journal.pone.0000897.