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Blue Whale (*Balaenoptera musculus*) Skin Contains Eumelanin and Pheomelanin

Blanca Morales-Guerrero,[1] Diane Gendron,[2] Laura M. Martinez-Levasseur,[3] and Karina Acevedo-Whitehouse[1, 4]

[1]*Unit for Basic and Applied Microbiology, School of Natural Sciences, Autonomous University of Queretaro, Avenida de las Ciencias s/n Juriquilla, 76230 Queretaro, México*
[2]*Laboratorio de Ecología de Cetáceos, Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional, Avenida IPN s/n Colonia Playa Palo de Santa Rita, 23096 La Paz, Baja California Sur, México*
[3]*Department of Biology, Trent University, Peterborough, ON, K9J 7B8, Canada*
[4]*The Marine Mammal Center, 2000 Bunker Road, Sausalito, CA 94965, USA*
E-mail: karina.acevedo.whitehouse@uaq.mx

Abstract

Melanin is a widespread pigment of the animal tegument. Two variants of melanin exist: (1) eumelanin, a dark brown pigment, and (2) pheomelanin, a reddish-orange pigment. While eumelanin is photoprotective, pheomelanin has been linked to cellular damage and high cancer risk. Despite this negative effect, pheomelanin is present in many, but not all, species, suggesting that it could confer an evolutionary advantage. To date, it is unknown whether the cetacean epidermis contains both melanin variants. Herein, we implemented a simple technique, previously developed for bird feathers, to quantify eumelanin and pheomelanin in blue whale (*Balaenoptera musculus*) skin, and we explored the potential biological role of pheomelanin based on histological analysis and gene expression quantitation. Both melanin variants were observed in the epidermis, with eumelanin being 42% more abundant than pheomelanin. Blue whale skin pigmentation or mottling type was predicted by the ratio of eumelanin to pheomelanin (EPR), with darker whales showing a higher EPR. Tyrosinase transcription levels influenced the EPR, with higher transcription being associated with higher EPR. Neither transcription of tumor suppressor gene p53 nor occurrence of epidermal photo-damage were related to the concentration of melanin variants, although there appeared to be a trend in which whales with blisters and microvesicles tended to have a lower EPR. Our study expands current understanding of epidermal pigmentation and photoprotection of large cetaceans.

Key Words: blue whale, *Balaenoptera musculus*, melanin, eumelanin, pheomelanin, pigmentation, photoprotection

Introduction

Melanin is an important photoprotective pigment due to its ability to absorb and disperse 50 to 70% of the solar ultraviolet (UV) radiation that penetrates the epidermis (Brenner & Hearing, 2008; Coelho et al., 2009). This pigment is synthesized by melanocytes, which are specialized skin cells that produce two different melanin variants: (1) the dark-brown eumelanin and (2) the reddish-orange pheomelanin. Both differ in their chemical structure and biosynthesis pathways (Yamaguchi et al., 2007). Photoprotective capacity also varies between the pigments, with eumelanin known to confer better photoprotection than pheomelanin (Brenner & Hearing, 2008).

Eumelanin and pheomelanin are synthesized by the enzyme tyrosinase, so the amount of each variant produced is determined mainly by the catalytic activity of tyrosinase and the availability of cysteine (Sturm et al., 2001; Barsh, 2003; Lin & Fisher, 2007), a proteinigenic aminoacid that contains sulphur. In humans, the pigmentation of an individual's skin is not only dependent on the quantity of these pigments and the eumelanin to pheomelanin ratio (EPR; Hennessy et al., 2005), but also on the number, distribution, and shape of melanosomes (i.e., vesicles found in the epidermal keratinocytes that contain eumelanin and pheomelanin) (Rana et al., 1999; Sturm et al., 2001; Costin & Hearing, 2007).

Synthesis and storage of pheomelanin has been considered to be detrimental at the cellular and organismal levels (Brenner & Hearing, 2008), namely due to the increased reactive oxygen species (ROS) associated with its synthesis (Nasti & Timares, 2015). However, some have proposed that this pigment could have a dermoprotective

function under certain physiological conditions (Greco et al., 2009; Galván & Solano, 2015). Regardless of this possibility, pheomelanogenesis requires cysteine (Morgan et al., 2013) that is obtained from glutathione, a major antioxidant molecule and a crucial factor in nutrient metabolism and cellular regulation (Wu et al., 2004). Thus, synthesis of pheomelanin depletes glutathione stores in melanocytes, making them more vulnerable to oxidative stress and carcinogenesis (Morgan et al., 2013). In addition, after an external (e.g., solar radiation) or internal (e.g., oxidative stress) challenge, pheomelanin-derived photoproducts are produced. These photoproducts include hydrogen peroxide, superoxide anions, and ROS, which are associated with epidermal oxidative stress, mutations, and DNA damage (Lin & Fisher, 2007; Brenner & Hearing, 2008; Yamaguchi et al., 2008; Greco et al., 2009). In fact, pheomelanin produces at least five times more peroxidase than eumelanin after exposure to solar UV radiation (Takeuchi et al., 2004). Furthermore, pheomelanin induces the release of histamine, which contributes to skin erythema and edema following such exposure (Brenner & Hearing, 2008).

At the organism level, negative effects of high pheomelanin concentration have been studied in birds in which they reportedly reduce development of the bird's brain (Galván & Møller, 2011) and increase the risk of developing cataracts (Galván et al., 2012b). In humans, a higher ratio of pheomelanin to eumelanin has been associated with increased risk of melanoma (Hennessy et al., 2005; Lomas et al., 2008). Recent studies have found that pheomelanin is not exclusive to terrestrial mammals and birds as previously thought. Various insects (Galván et al., 2015; García et al., 2016), mollusks (Speiser et al., 2014), amphibians (Wolnicka-Glubisz et al., 2012), and reptiles (Roulin et al., 2013) also have this pigment, although pheomelanin has not been detected in the tegument of other organisms such as spiders (Hsiung et al., 2015) and fish (Ito & Wakamatsu, 2003). Its widespread distribution across taxa would suggest that in spite of its detrimental effects, pheomelanin is under positive selection. A possible explanation for its high prevalence is that under non-stressful cellular conditions, pheomelanin can act as a major cysteine excretory system, thus helping to avoid its toxic effects when it oxidizes (Galván & Solano, 2015). In fact, pheomelanin seems to have some intrinsic stability to UV radiation and ROS degradation (Greco et al., 2009).

Recent studies on the skin of three large whale species reported that the number of melanosomes is inversely related to UV-induced microscopic lesions such as microvesicles and cytoplasmic vacuolation (Martinez-Levasseur et al., 2011). In addition, the abundance of melanosomes in blue whales appears to be dependent on the number of melanocytes in the skin (Martinez-Levasseur et al., 2013). However, the presence and relative proportion of both melanin variants in the cetacean epidermis has yet to be investigated. We have implemented a technique to quantify eumelanin and pheomelanin in blue whale (*Balaenoptera musculus*) skin, and have explored the biological significance of these compounds. Determining whether pheomelanin is present in cetaceans will expand current knowledge about their epidermal physiology and might be relevant to better understand the increasing incidence of skin lesions in cetaceans around the world (e.g., Wilson et al., 1999; Van Bressem et al., 2009, 2014; Martinez-Levasseur et al., 2011; Hart et al., 2012).

Methods

Sample Collection

We quantified both melanin pigments in skin biopsies that had been collected from 26 apparently healthy adult blue whales in the Gulf of California, Mexico, between 2007 and 2009 (Figure 1). These biopsies had been used in a previous study on UV-induced damage of blue whales (Martinez-Levasseur et al., 2011). Briefly, samples were collected using a carbon fiber dart with a 7-mm stainless-steel cutterhead that was shot from a crossbow (see method explanation in Costa Urrutia et al., 2013). Immediately after collection, skin samples were sectioned and preserved in 10% buffered formaldehyde in aluminum foil-wrapped glass vials that were kept in a dark cooler to protect them from sunlight (see Martinez-Levasseur et al., 2011). Prior to collecting the skin biopsies, each whale was photographed using a digital camera (Canon EOS20) for individual identification (Gendron & Ugalde de la Cruz, 2012).

As the samples had been collected as part of a larger study on cetacean susceptibility to UV radiation, biopsies had been subsampled for different projects; and there was data available on various photographic, histological, and molecular aspects for a number of the samples. Namely, for 18 of the blue whales, information on the number of melanocytes and melanosomes per epidermal section was available (Martinez-Levasseur et al., 2011); and for 25 of the blue whales, we had photographic and histological data on the presence of gross blisters, cytoplasmic vacuolation, microvesicles, and intracellular edema, which commonly occur during acute sunburn in humans (Nakaseko et al., 2003) and have been associated with exposure to UV radiation in these whales (Martinez-Levasseur

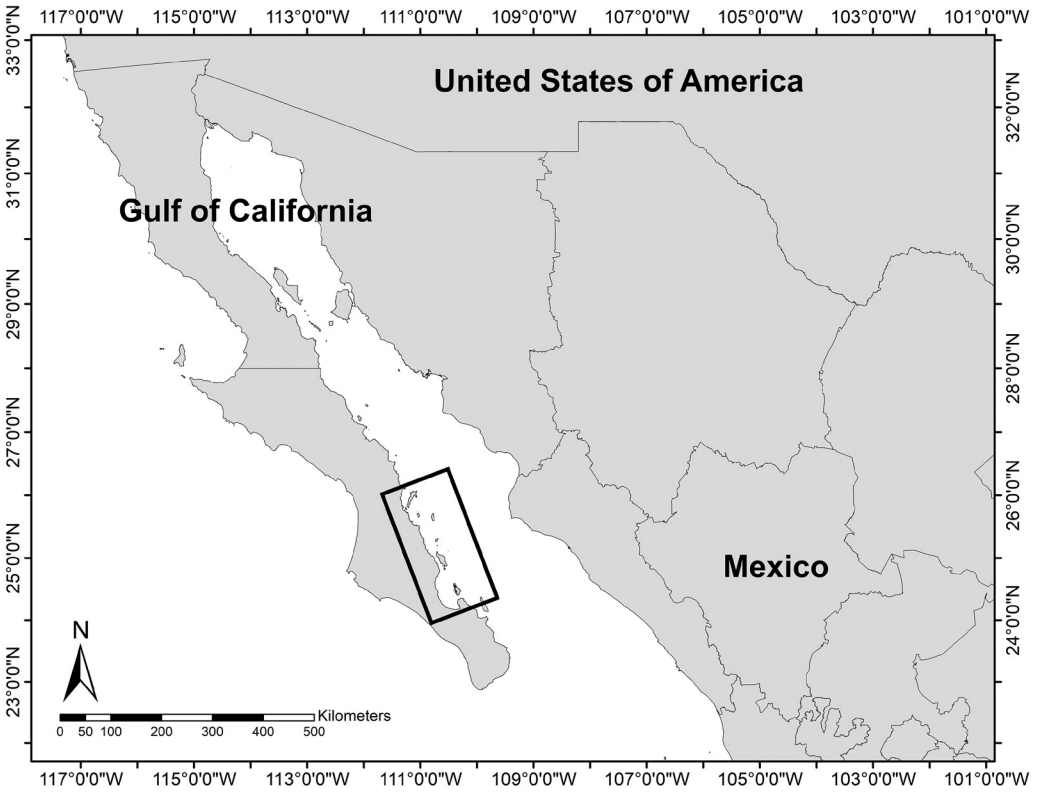

Figure 1. Map of the Gulf of California; the black rectangle shows the study area where samples were collected.

et al., 2011). We also had data on the relative transcription levels of tyrosinase (TYR) and tumor suppressor protein (p53) genes in skin cDNA (Martinez-Levasseur et al., 2013) for a subset of the samples ($n = 9$). Finally, for 13 of the blue whales, we had data on the numerical value of their skin pigmentation type (hereafter, skin color value), which had been obtained by digital analysis of high-quality photographs of their dorsolateral surface (see details below).

Estimation of Skin Color Value

A numerical value of skin coloration was determined for the individual blue whales based on lateral photographs taken with a digital camera (Canon EOS20) before or after remote biopsy sampling. Initial criteria for inclusion of the photographs included being in focus, having minimal angle to the camera, and having no back illumination nor dim lighting (Urian et al., 2015). Selected photos were uploaded in *GNU Image Manipulation Program* (*GIMP2*; license *GPLv3*; www.gimp.org) and were converted to a scale of grey to facilitate the measurement of color. We faced two problems. First, despite the majority of the biopsies being taken above the midline in the dorsal flank (i.e., cranial to the dorsal fin), it was not possible to pinpoint in the photograph the exact location where the biopsy was collected for every individual. Second, the dorsal flank was not uniform in color; generally, the upper dorsal flank exhibited a darker pigmentation than the lower area (Paired t test: $t = 2.622$, $df = 12$, $p = 0.022$; see Figure 2). Even if the remote biopsy dart normally sampled the upper dorsal flank, it was possible that for some individuals, the remote biopsy dart would have sampled the lower (lighter) dorsal flank. Thus, we developed a protocol that took these issues into account. For each photograph, we drew six matrices of 150 × 150 pixels (px), yielding an area of 135,000 px. Three matrices were drawn from the beginning of the dorsal fin towards the head, and three matrices were drawn starting from where the proximal edge of the third top matrix ended (Figure 2).

Only photographs for which it was possible to draw the above-mentioned matrices were included in the analysis. The frames resulting from the six

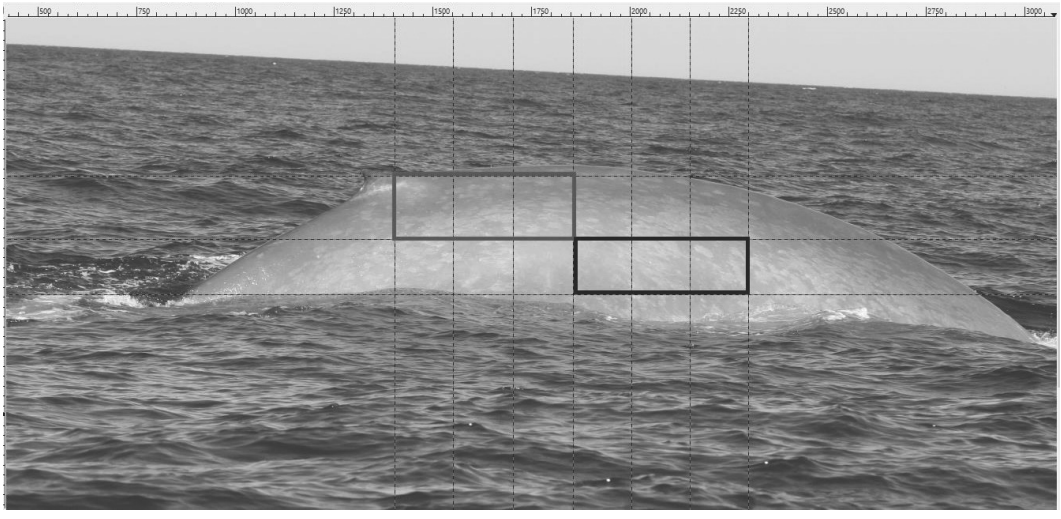

Figure 2. Location of matrices where the individual color value of whales was determined. As can be seen, the upper dorsal flank is darker than the lower dorsal flank. The dark grey rectangle shows the three matrices in the darker upper dorsal area, while the black rectangle shows the three matrices in the lighter lower area.

drawn matrices were selected, cut, and exported to *Paint 6.2* (Microsoft, Redmond, WA, USA). The quadrant was adjusted to the trimmed frame to exclude any white areas outside the frame that would affect the results. Measurement of individual coloration was performed using the 'ReadImages' package of *R*, Version 2.3.2, to assign a numerical value to the color of each px. A value of 1 was absolute dark, and 0 was absolute white. In this way, six numerical matrices were generated for each photograph. To minimize errors derived from subtle variations in the ambient light, a correction factor was applied to each of the matrices with the following equation:

$$\mathrm{SC} = Log_2\left(\frac{f^2}{t}\right) \times \Sigma \mathrm{nvc}$$

where f is the diaphragm aperture of the camera, t the exposure time, and Σnvc is the sum of the numerical value of the color of each px that composed the matrix. The SC calculated for each of the three matrices was averaged to yield a single numerical value for each photograph.

Quantitation of Skin Pigments

To detect and quantify eumelanin and pheomelanin, we used a simple low-cost patented method (p200703395 in the European Union), which was developed originally for bird feathers and has been used for tissues from other animals (Galván et al., 2012a). As the method was developed for feather samples, we modified the protocol to optimize it for blue whale skin. Biopsy samples were first weighed in an HRB-203 precision scale (TREE Balances; LW Measurements LLC, Santa Rosa, CA, USA) with a precision of 0.001 g and then washed with 50 µL of phosphate buffered saline (PBS) for 1 min prior to cutting the sample into small sections with a sterile scalpel blade and crushing them with a glass pestle. To avoid inter-sample transfer, the pestle was washed with NaOH 0.1N, rinsed with distilled water, and dried with a fresh paper towel between samples.

We performed an alkaline digestion of the skin sections with 1 ml of 20% NaOH. Samples were placed in a sonication water bath at 60ºC for 40 min to complete sample digestion. The soluble pheomelanin fraction was separated from the eumelanin fraction by centrifugation at 17,500 g for 15 min at 4ºC. The supernatant, which contained soluble pheomelanin, was transferred into a new microtube, and its absorbance was measured at 450 and 600 nm in a spectrophotometer, using NaOH at 20% as a blank. The arbitrary units of pheomelanin per sample (AU) divided by sample mass were calculated as follows:

$$AU = \frac{A_{450} - A_{600}}{W}$$

where A_{450} is the absorbance measured at 450 nm, A_{600} is the absorbance at 600 nm, and W is the mass of the skin sample (mg) (Negro et al., 2009).

Next, we quantified eumelanin. For this, we added 1 ml of 20% NaOH to the black pellet from the previous step and resuspended it by agitation prior to precipitating the sample by centrifugation

at 17,500 g for 15 min at 4ºC. The supernatant was removed, and the pellet was resuspended and precipitated once more as indicated above. Once the supernatant was removed, the pellet was dried at 60ºC. We added 1 ml of 20% NaOH and 20 µl of 30% H_2O_2 to oxidize the eumelanin. The mixture was vigorously mixed with a vortex shaker and placed in a sonicated bath at 60ºC for 10 min. The oxidation reaction was stopped by adding 50 µl of 40% $NaHSO_3$. The samples were centrifuged at 17,500 g for 15 min at 4ºC, and the absorbance at 450 and 600 nm were measured immediately. The arbitrary units of eumelanin per skin sample mass were calculated as indicated above. The method was repeatable as observed from processing and measuring values in duplicate in a set of six samples (eumelanin concentration: $r^2 = 0.88$, $F_{1, 4} = 31.80$, $p = 0.005$; pheomelanin concentration: $r^2 = 0.86$, $F_{1,4} = 26.55$, $p = 0.007$).

We calculated the total amount of melanin pigments per individual by summing the value recorded for both variants. Finally, we calculated the eumelanin to pheomelanin ratio (Nakagawa & Imokawa, 1996; Hennessy et al., 2005; hereafter EPR). This ratio is a measure of the relationship between both melanin variants. In humans, the EPR is related to an individual's skin color value and to the degree of microscopic skin damage following exposure to solar UV radiation (Hennessy et al., 2005).

Statistical Analyses

We initially explored our dataset graphically to establish the spread and distribution of each variable. Continuous response variables were examined with Shapiro-Wilk tests, and equality of variance was assessed with Levene's test. Skin color value, EPR, and the total amount of melanin conformed to the expectations of a normal distribution, while eumelanin and pheomelanin concentrations revealed a slightly right-skewed distribution and were log-transformed to achieve normality (Shapiro Wilk normality test: eumelanin: $W = 0.96$, $p = 0.496$; pheomelanin: $W = 0.95$, $p = 0.272$). We compared the means of eumelanin and pheomelanin in the skin biopsies with a two-tailed *t* test. We calculated Pearson's correlation coefficient to explore whether the concentrations of both melanin variants were related as occurs in human skin (Hennessy et al., 2005).

We used linear regressions to examine whether the number of melanocytes and melanosomes predicted the total amount of melanin pigments in a skin biopsy. We also used linear regressions to examine whether a whale's skin color value (response variable) was determined by the amount of melanin pigments—eumelanin, pheomelanin, and EPR, in turn.

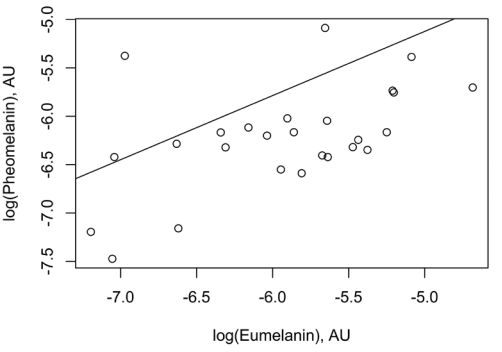

Figure 3. Relationship between the concentrations of eumelanin and pheomelanin in blue whale skin biopsies. Eumelanin and pheomelanin concentrations are shown as log-transformed values of the arbitrary units per skin sample mass.

We explored the biological relevance of the epidermal pigments by using linear and logistic regressions. First, we investigated whether the relative transcription levels of TYR helped explain the total amount of melanin pigments (sum of eumelanin and pheomelanin) and the EPR. We next explored whether tumor suppressor gene p53 transcription was predicted by EPR. This research question was based on the fact that a previous study found that blue whale p53 transcription levels increased according to the UV radiation index (Martínez-Levasseur et al., 2013), and this gene is known to be involved in DNA repair, cell cycle arrest, and apoptosis in response to stressors, including UV radiation (Latonen & Laiho, 2005). For the logistic regression models, the presence (or absence) of previously identified lesions (e.g., gross blisters, cytoplasmic vacuolation, microvesicles, and intracellular edema) was modeled as the response variable, while EPR, eumelanin, and pheomelanin were modeled as explanatory variables. As we had a modest number of data points, each model included only one explanatory variable at a time. For all statistical analyses, significance was considered at an alpha level of 0.05. *R*, Version 3.3.2 (R Development Core Team, 2016) was used to run all analyses and create all graphs.

Results

The total amount of melanin pigments per sample averaged 0.0053 (± 0.0024 SD). Eumelanin and pheomelanin were detected in all of the skin biopsies. The mean concentration of eumelanin (0.0032) was 1.42 times higher than that of pheomelanin (0.0022) (two-tailed *t* test: $t = -2.06$, $df = 42.28$, $p = 0.045$). Inter-sample variation (SD) was 0.0019 for eumelanin and 0.0013 for pheomelanin.

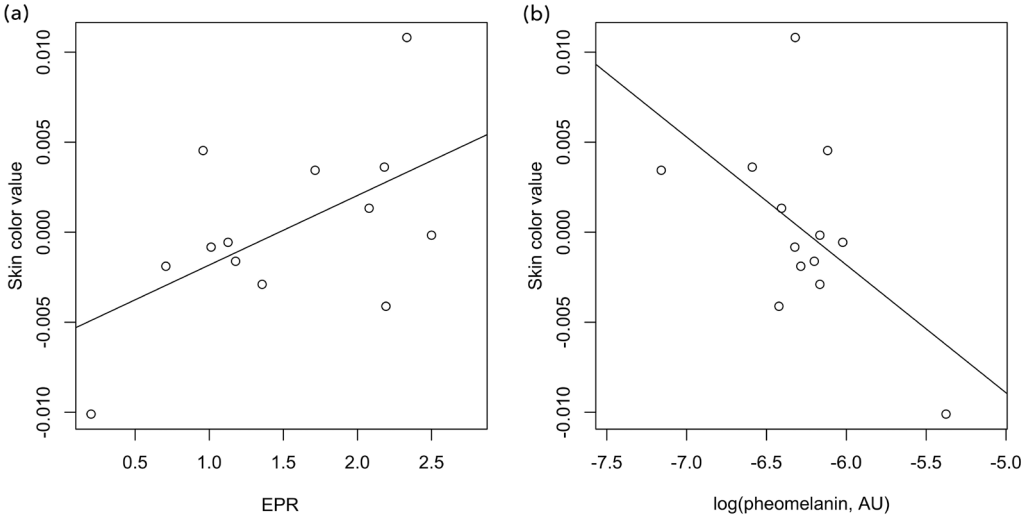

Figure 4. Blue whale skin color value is influenced by melanin pigments: (a) Relationship between the eumelanin to pheomelanin ratio (EPR) and the color value of the skin, and (b) relationship between the concentration of pheomelanin (log [arbitrary units per skin sample mass]) and the color value of the skin.

The concentrations of eumelanin and pheomelanin were highly correlated (Pearson's correlation = 0.53, $t = 3.07$, $df = 24$, $p = 0.005$; Figure 3).

The total amount of melanin was not related to the number of melanocytes (R^2 adj. = -0.05, $df = 16$, $p = 0.722$) or the number of melanosomes (R^2 adj. = -0.06, $df = 16$, $p = 0.839$). Skin color value was predicted by its EPR (R^2 adj. = 0.24, $df = 11$, $p = 0.049$; Figure 4a) and pheomelanin concentration (R^2 adj. = 0.25, $df = 11$, $p = 0.047$; Figure 4b) but not by its eumelanin concentration ($p = 0.128$).

Transcription levels of TYR predicted the EPR, with whales with higher transcription levels of TYR tending to have higher EPR (R^2 adj. = 0.34, $df = 8$, $p = 0.05$; Figure 5). However, TYR transcription levels did not explain the total amount of melanin pigments in the skin sections ($p > 0.05$).

None of the markers of epidermal damage nor p53 transcription levels were related to the concentrations of either of the melanin variants or to the EPR (in all cases, $p > 0.05$). However, there was an apparent but nonsignificant trend in which whales with gross blisters, microvesicles, and intracellular edema tended to have lower EPR (Figure 6).

Discussion

Pheomelanin is known to be absent in most marine organisms, including fishes (Ito & Wakamatsu, 2003). As mammals, it would be expected that cetaceans would present both kinds of pigments in their skin. However, given that some of the photoprotective traits that this taxonomic group exhibit have not been detected in terrestrial mammals (Morales-Guerrero et al., 2017), and that other vertebrates that inhabit the marine environment, such as marine fishes, do not appear to have pheomelanin (Ito & Wakamatsu, 2003), it was possible that whale skin would not contain pheomelanin. Based on our observations, it appears that despite inhabiting the oceans for at least 50 million years (Berta et al., 2015), blue whales preserved pheomelanin, suggesting that pheomelanin was present in their common terrestrial ancestor.

In humans and other terrestrial mammals, skin color appears to be partially determined by the EPR of an individual (Rana et al., 1999; Hennessy et al., 2005; Yamaguchi et al., 2007; Wolnicka-Glubisz et al., 2012; Roulin et al., 2013; Speiser et al., 2014; Galván et al., 2015; García et al., 2016), and we found a similar pattern. Pheomelanin concentrations and the ERP explained 25% of the variation in skin color value, regardless of the number of melanosomes in the skin section. Thus, although other factors could influence skin color, the relationship between skin color and melanin pigments certainly appears to be important for the blue whale. For instance, age and seasonal variation could certainly influence skin color (Martinez-Levasseur et al., 2011, 2013). However, our limited sample size precluded further exploration of this possibility. Future studies that target animals across different age classes and seasons could provide additional information to build upon our findings.

Despite a previous study having demonstrated a positive association between the number of melanocytes and the quantity of melanosomes in 115 large whales, including blue whales (Martinez-Levasseur et al., 2011), we failed to find a relationship between any of these variables and the total amount of melanin pigments synthesized. It is possible that the apparent discrepancy between both studies is due to the difference in the sample sizes and/or the methods used to measure pigmentation. While the previous study inferred the amount of melanin by counting melanosomes, we quantified both epidermal melanin pigments directly. The lack of an association between melanocytes and the total amount of melanin concurs with what has been reported for humans for whom the number of melanocytes remains equal regardless of skin color; and, thus, it is melanogenic activity that is responsible for variations in skin color (Barsh, 2003; Brenner & Hearing, 2008). Furthermore, rather than the number of melanin-containing vesicles per se, it is the distribution and shape of melanosomes that has an influence on human skin pigmentation (Costin & Hearing, 2007; Brenner & Hearing 2008). It was not possible to examine differences in the distribution and shape of melanosomes in the whale skin biopsies with the samples available for our study. Future work should aim to explore this possibility.

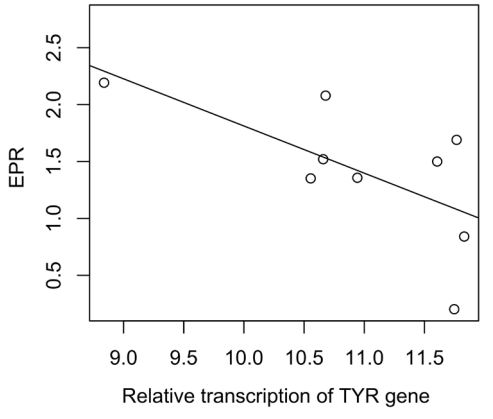

Figure 5. Relationship between transcription levels of TYR and the EPR

Genetic factors also play a role in shaping the amount of eumelanin and pheomelanin produced. The TYR gene, in particular, is known to play a key role in the initiation of melanin synthesis (Oetting, 2000; Sturm et al., 2001; Barsh, 2003; Lin & Fisher, 2007; Yamaguchi et al., 2007). Interestingly, despite our modest sample size, we found that levels of TYR transcription explained variation in the EPR, but TYR transcription levels did not predict the total amount of melanin pigments. It would appear that for blue whales, TYR influences the type of pigment synthesized, as has been described for humans (Oetting, 2000; Sturm et al., 2001; Barsh, 2003; Lin & Fisher, 2007; Yamaguchi et al., 2007), but other genes, such as tyrosinase-related protein 1 (TYRP-1), dopachrome tautomerase (DCT; also known as tyrosinase-related protein 2), premelanosome protein (PMEL), melanocortin receptor (MC1R), and membrane-associated transporter (SLC45A2) might play a role in cetacean melanogenesis as occurs in other species (Braasch et al., 2007; Deng & Xu, 2017). Furthermore, post-transcriptional modification of TYR can also influence the production of melanin pigments (Okamura

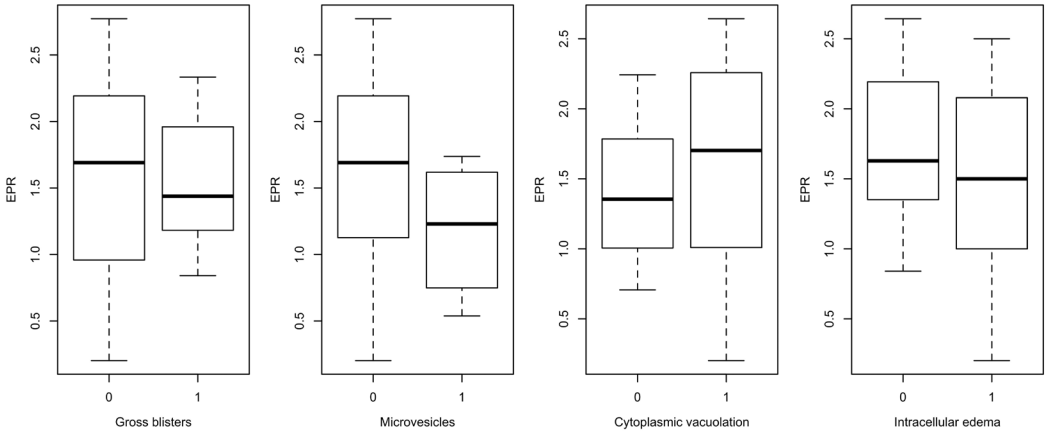

Figure 6. EPR in blue whale skin biopsies with (1) and without (0) UV-related lesions; the bold lines show the median responses, the boxes encompass the quartiles, and the whiskers indicate the endpoint data.

et al., 2017), further complicating the relationships between gene transcription and melanin synthesis. In addition, the catalytic activity of TYR can be determined by the allelic variants of this gene (Sata et al., 2000). While well known for humans, little is known about TYR polymorphism in cetaceans, although one study reported that skin color variants such as mottled skin and albinism are related to allelic variations of TYR in the humpback whale (*Megaptera novaeangliae*; Polanowski et al., 2012). To the best of our knowledge, studies on genetic polymorphisms of TYR have yet to be conducted in blue whales. Such studies should aim to investigate if allelic variants of TYR exist in the population and whether they influence the type and amount of pigment synthesized in the blue whale.

In terms of investigating the photoprotective role of eumelanin and the harmful role of pheomelanin in blue whale skin, we had data, albeit limited, with which to explore such potential biological significance. In humans, UV-related lesions, such as microvesicles, cytoplasmic vacuolation, edema, and leukocyte infiltration are more common in individuals with lower EPR (see Brenner & Hearing, 2008), and we expected to find a similar pattern based on previous observations that show that UV-related lesions are prevalent in blue whales (Martinez-Levasseur et al., 2011). It is possible that in blue whales, pheomelanin does not exert a detrimental effect. In fact, the widespread presence of this melanin variant in different species implies that it is not necessarily under negative selection (Galván, 2017). However, our failure to find a significant relationship between the ratio of both melanin variants and UV-associated damage could arise because cellular responses can have a marked individual component (Westerhof et al., 1990), which is related to factors such as senescence (Kammeyer & Luiten, 2015), physiological status, and immune activity (McKee et al., 2014). However, despite the lack of statistical significance, plausibly driven by our modest sample size, there was an apparent pattern in which whales that had gross blisters and microvesicles, previously linked to UV-related lesions (Martinez-Levasseur et al., 2011), tended to have lower EPR. If this pattern were to be confirmed with a larger sample size, it would imply that individuals with higher pheomelanin concentrations would be more likely to have epidermal damage due to solar UV radiation as occurs in humans (Takeuchi et al., 2004), and the EPR could be considered an index to predict susceptibility to UV-related damage in blue whales. Future studies that include more individuals across age classes could help to understand the functional significance of cetacean pheomelanin better. Potentially, older whales would have been exposed to more UV over the course of their lives, and, presumably, UV-repair mechanisms would be less efficient; thus, in these animals, the EPR could be linked to UV-associated damage more clearly.

In conclusion, our study has shown that both melanin variants are synthesized in the blue whale epidermis and that skin color is influenced by the proportion of both melanin variants, similar to what occurs in other organisms (Rana et al., 1999; Yamaguchi et al., 2007; Wolnicka-Glubisz et al., 2012; Roulin et al., 2013; Speiser et al., 2014; Galván et al., 2015; García et al., 2016). To the best of our knowledge, ours is the first study to identify and quantify pheomelanin in the skin of any cetacean and, thus, adds to our understanding of cetacean melanogenesis. However, taking into consideration that cetacean photoprotective strategies appear to be species-specific (Martinez-Levasseur et al., 2013; Morales-Guerrero et al., 2017), similar studies should be conducted across a range of species to fully understand the biological significance of cetacean eumelanin and pheomelanin.

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Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Sampling of all whales had been conducted between 2007 and 2009 by DG and LMM under permit numbers SGPA/DGVS/00506/08, SGPA/DGVS/09760/08, and SGPA/DGVS/08021/06, which were issued by the Mexican Secretary of the Environment. Sampling was conducted as part of research projects SIP 20060945, 20070803, and 20080846 at the Instituto Politecnico Nacional, and WLE/0474 at the Institute of Zoology, and was approved by the Bioethics Committee of the Zoological Society of London. Our study was approved by the Bioethics Committee of the School of Natural Sciences at the Autonomous University of Queretaro. All procedures adhered to guidelines stipulated by national and international laws of animal research, where animal handling and sampling were undertaken, and to those outlined by the Autonomous University of Queretaro in their ethics statement on the use of animals for research and teaching.

Author Contributions

BMG and KAW conceived the idea and wrote the manuscript. BMG performed eumelanin and pheomelanin quantitation, calculation of the numerical value of skin color, and statistical analyses. DG and LMM obtained the skin biopsies. DG supervised fieldwork and conducted photographic sampling of the whales. LMM performed the gene transcription quantitation assays and histological assessment of skin biopsies. All authors discussed the results and commented on the manuscript.

Data Availability

The datasets used for the current study are available from the corresponding author on reasonable request.

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