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Melanin granules melanophages and a fully-melanized epidermis are common traits of odontocete and mysticete cetaceans

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Background – The cellular mechanisms used to counteract or limit damage caused by exposure of marine vertebrates to solar ultraviolet (UV) radiation are poorly understood. Cetaceans are vulnerable because they lack protective skin appendages and are obliged to surface continuously to breathe, thus being exposed repeatedly to UV light. Although molecular mechanisms of photoprotection of cetaceans have been studied, there is limited knowledge about their epidermal structure and photoprotective effectors.

Objective – To describe and compare the epidermis of mysticete and odontocete cetaceans and identify potentially photoprotective traits.

Animals – Twenty eight free-living individuals belonging to six cetacean species were sampled in the Mexican Central Pacific and Gulf of California. Species sampled were the bottlenose dolphin, pantropical spotted dolphin, spinner dolphin, Bryde's whale, fin whale and humpback whale.

Methods – Histological and cytological evaluation of skin biopsy tissue collected in the field between 2014 and 2016.

Results – All cetaceans had only three epidermal layers, lacking both the stratum granulosum and stratum lucidum. A relatively thick stratum corneum with a parakeratosis-like morphology was noted. Melanin was observed within keratinocytes in all epidermal layers, including the stratum corneum and apical melanin granules obscured the keratinocyte nucleus. Keratinocytes had a perinuclear halo. Keratinocyte diameter differed between cetacean suborders and amongst species. Melanophage clusters were common in most cetacean species.

Conclusions – The widespread presence of melanin and the unexpectedly high number of melanophages may constitute a unique photoprotective trait of cetaceans and could reflect primitive adaptations to their environment and to their obligate marine-bound life.

Introduction

Solar ultraviolet radiation (UV) is one of the most damaging agents that exist on our planet. In the past few decades, concerns about the effects that UV can have on aquatic environments have increased in the scientific

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community.^{1–3} UV is known to affect organisms from almost all trophic levels, including phytoplankton,⁴ zooplankton,⁵ amphibians,⁶ sea urchins,^{7,8} coral,² starfish⁵ and fish.^{7,9} Studies have demonstrated evidence of acute sunburn¹⁰ and mitochondrial DNA damage in large whales.¹¹ Considering that cetaceans are repeatedly exposed to UV when they surface to breathe or socialize, lack protective fur coats and cannot avoid exposure,^{12,13} it is pertinent to increase our understanding of cetacean skin structure and its potential photoprotective mechanisms.

In terrestrial mammals, the epidermis is composed of five distinct layers that vary in structure depending on anatomical location, season and life history stage.^{14,15} In cetaceans, an obligate aquatic life has led to a series of epidermal modifications such as lack of hair and other

structural differences.^{16–19} For example, fin whales (*Balaenoptera physalus*) have only three epidermal strata instead of the five strata common to other mammals.^{17,20} Furthermore, cetacean species appear to have an exceptionally thick epidermis, being 15 to 20 times thicker than that of terrestrial mammals.^{18,21}

Mammalian skin has different photoprotective mechanisms studied predominantly in humans and laboratory model animals. The most abundant photoprotective effector is melanin, a pigment produced by melanocytes that acts as a UV-absorbing compound located in the deepest layers of the mammalian epidermis.^{22–27} Melanin is transferred actively from melanocytes to keratinocytes in the basal layer and stratum spinosum within organelles termed melanosomes.^{23,27–29} Melanin also protects basal keratinocytes from UV and avoids damage to the underlying connective tissue, a process achieved by supranuclear relocation of melanin.^{1,23,24}

Studies evaluating cetacean photoprotection at the molecular and cellular levels would suggest some evidence of distinct strategies between species.^{11,30} Blue whales (*Balaenoptera musculus*) increase the transcription of genes related to melanin production, whereas sperm whales (*Physeter macrocephalus*) overexpress genotoxic stress effectors in response to UV.³⁰ Furthermore, blue whales with increased melanin tend to have less sunburn lesions and higher levels of apoptosis.¹¹ Despite this information, basic knowledge regarding cetacean skin histology and its photoprotective role remains limited.

This study describes cetacean skin, and compares epidermal structure, cellular morphology and photoprotective effectors between suborders and across species. Epidermal skin sections of six mysticeti and odontoceti cetacean species were evaluated. Our aim was to generate data for future investigation of the ecological and evolutionary constraints of photoprotective effectors in cetacean skin.

Material and methods

Skin biopsies from the dorsal surface (posterior to the dorsal fin) were collected from individuals of six cetacean species from the Mexican Central Pacific coast during November 2014 and March 2015, and in the Gulf of California during February 2016. Odontocete species sampled included the bottlenose dolphin (Tursiops truncatus), the pantropical spotted dolphin (Stenella attenuata) and the spinner dolphin (S. longirostris); mysticete species sampled were the Bryde's whale (Balaenoptera edeni), B. physalus and the humpback whale (Megaptera novaeangliae). Skin biopsies were collected using a crossbow and arrow with a modified biopsy tip.31 For histological analyses three individuals of each species were examined except for the Bryde's whale, for which only one sample was available. For cytological analyses, a total of 28 skin cell smears were examined from nine spinner dolphins, five pantropical spotted dolphins, seven bottlenose dolphins, three humpback whales, three fin whales and one Bryde's whale.

Impression smears of skin biopsy tissue were collected on a slide and then spray-fixed (Fija-cell[®], Materiales y Abastos Especializados; Zapopan, Jalisco, México). Tissue was then preserved in 10% buffered formalin. Formalin fixed samples were paraffin embedded and a microtome used to cut 3 μ m sections that were mounted and stained with haematoxylin and eosin (H&E). The 16 H&E skin sections were examined under light microscopy (10×, 40× and 100×) and epidermal structure, melanin distribution and cell dimensions $(40\times)$ were determined. For each skin slide the width and length of ten cells selected at random per stratum were determined in order to calculate the cell area (A), using the following equation:

$$A = \pi \left(\frac{a}{2} \times \frac{b}{2}\right)$$

where *a* and *b* correspond to the length and width of the cell, respectively.

Skin biopsy tissue was evaluated for lesions described previously for UV-induced damage in whales (cytoplasmic vacuolation, intracellular oedema or glycogen deposition.)¹¹ Cell smears were stained with a modified Papanicolau stain and examined under light microscopy $(40\times)$.³² This approach allowed us to identify and count each cell type. Cellularity was determined for each cell type as the percentage of cells relative to the total number of cells in the smear, and was standardized per mm².

All variables were examined for deviations from normality. Oneway ANOVAs and *post-hoc* Tukey Honest Significance Difference test (HSD) tests were used to investigate interspecies differences in the intensity of melanin, cell diameter and cell counts (for each cell type, n = 10 cells per individual). Kruskal–Wallis (KW) tests were used to examine interspecies differences in the numbers of melanophages and lymphocytes, and we further examined differences using *post hoc* Tukey HSD tests. In all cases, statistical significance was set at P < 0.05. All analyses were conducted in R 2.14.0 (R Core Team. R: A Language and Environment for Statistical Computing. <http://www.R-project.org/>. (R Foundation for Statistical Computing, Vienna, Austria, 2015)).

Results

All cetacean species evaluated had only three of the five epidermal strata described for terrestrial mammals, with the stratum granulosum and stratum lucidum being absent. The basal stratum flanked the dermal papillae and comprised predominantly keratinocytes and few melanocytes. Basal keratinocytes were oval shaped with the major axis perpendicular to the edge of the dermal papilla with a reduced cytoplasm and a prominent nucleus. The stratum spinosum was composed of keratinocytes with a large oval nucleus and moderate amounts of cytoplasm. Closer to the epidermal surface, keratinocytes had a more flattened appearance with a reduced nuclear volume. For all individuals, melanin was observed within cells in all of the epidermal layers, even in the stratum corneum (Figure 1). The stratum corneum was relatively thick (resembling hyperkeratosis) and was composed of elongated and flattened keratinocytes with a small hyperchromatic nucleus. Many cells retained their nuclei (i.e. parakeratosis-like morphology).

From the basal layer to the stratum corneum, keratinocytes typically had a perinuclear halo, and melanin granules were present in the keratinocyte cytoplasm, including those in the stratum corneum. Granules were arranged apically within the cells and formed an "umbrella-like" structure that covered the keratinocyte nucleus (Figure 2). The external keratin layer retained melanin granules. There was diffuse epidermal thickening resembling epidermal hyperplasia (acanthosis) and the dermal papillae were long, encompassing almost half of the total epidermal thickness. Melanocytes were slightly smaller than the basal keratinocytes; their cytoplasm and nucleus were obscured by high numbers of melanin granules and the nuclei were markedly hyperchromatic.



Figure 1. Microphotograph of the epidermis of a spotted dolphin (*Stenella attenuata*). The fully melanized epidermis was observed in all individuals of all species examined. Haematoxylin and eosin; scale bar 150 µm.



Figure 2. Microphotograph of the stratum spinosum of a fin whale (*Balaenoptera physalus*). Perinuclear halos are evident, see white arrow head. (A). Melanin granules can be observed surrounding the keratinocyte nucleus apically, see white arrow. (B). Haematoxylin and eosin; scale bar 50 μm.

The number of keratinocytes did not vary amongst species (KW, $\chi^2 = 10.244$, df = 5, P = 0.069). However, keratinocyte diameter differed between mysticetes and odontocetes (F_{1,14} = 44.03, $P = 1.12 \times 10^{-05}$) and also amongst species within both suborders (see Table 1; for mysticete species: F_{2,4} = 20.53, P = 0.00788; for odontocete species: F_{2,6} = 6.382, P = 0.0327). Differences in keratinocyte diameter were most pronounced in the basal layer (F_{5,10} = 39.13, $P = 3 \times 10^{-06}$) and the stratum

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spinosum (F_{5,10} = 10.29, *P* = 0.001) but were absent in the stratum corneum (F_{5,10} = 3.261, *P* = 0.053). Melanocytes were not observed in any of the cell smears, most likely due to their strong adhesion to the epidermal basal layer. In the skin sections, the number of melanocytes differed between mysticetes and odontocetes (F_{1,14} = 5. 198, *P* = 0.0388), and they varied amongst odontocete (F_{2,6} = 61.2, *P* = 0.0001), but not mysticete species (F_{2,4} = 2.385, *P* = 0.208; see Table 2).

The number of epidermal lymphocytes remained constant among species (KW, $\chi^2 = 5.581$, df = 5, P = 0.349). However, large numbers of melanophages were observed in most of the individuals (two of three fin whales, one of three spotted dolphins, one of three bottlenose dolphins and all three humpback whales examined). Melanophage counts varied amongst species, being highest in the humpback whale (KW, $\chi^2 = 16.939$, df = 5, P = 0.005; *post hoc* Tukey HSD test: $P = 1 \times 10^{-04}$). Melanophages always appeared as cell clumps composed of two to hundreds of cells per aggregate.

Discussion

The absence of the stratum granulosum and stratum lucidum, and the parakeratosis-like morphology of the epidermis have been described previously in cetacean species.^{17–19} Acanthosis and parakeratosis were observed in all individuals from both mysticeti and odontoceti groups in the absence of inflammatory response or cellular damage. Epidermal acanthosis and parakeratosis often are observed in chronic inflammatory skin conditions of humans,²⁹ but we presume that these are normal components of the cetacean epidermis. It is feasible that these could reflect primitive adaptations to an aquatic environment with sustained exposure to UV.^{11,19}

Keratinocyte diameter was three to five times larger in cetaceans than humans,³³ and varied between and within mysticete and odontocete species. Although keratinocyte diameter was larger in mysticetes than in odontocetes, body size is unlikely to explain variation because the largest keratinocyte diameter was observed for the Bryde's whale, which is not the largest species studied (of the study species, fin whales are the largest with 22 m body length).³⁴ It is likely that variation in keratinocyte size could be explained by other life history or phylogenetic factors.

Additionally keratinocytes had an apical accumulation of melanin above the cell nucleus in all cetaceans studied. This trait has been reported previously for melanocytes of blue whales, sperm whales and fin whales.¹¹ Keratinocyte realignment of melanin has been described in detail in humans and is known to constitute a protective response against UV.14,24 It is feasible that cetaceans also rely on this mechanism for photoprotection. However, despite melanin's capacity for UV attenuation and dispersion in the skin, protection afforded by this pigment during prolonged or repeated exposure to UV is somewhat limited.²⁸ Cetaceans have an obligate aerobic physiology, and activities such as nursing and socializing result in prolonged periods at the sea surface and subsequent acute UV damage.²¹ Acute sunburn has been reported for blue, sperm and fin whales from the Gulf of California,

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Table 1. Keratinocyte diameter (nm²) of mysticete and odontocete cetacean species. Values shown are mean and SD.

Species	All layers	Basal layer	Stratum spinosum	Stratum corneum
Mysticetes	375.85 (51.34)	267.74 (61.80)	493.27 (110.20)	366.54 (37.21)
Bryde's whale	468.81 (NA)	335.46 (NA)	244.55 (NA)	312.17 (NA)
Fin whale	333.49 (53.75)	224.69 (16.61)	417.18 (29.43)	358.60 (53.75)
Humpback whale	387.23 (53.33)	269.54 (27.25)	499.53 (26.39)	387.23 (53.33)
Odontocetes	229.25 (37.22)	137.12 (66.32)	309.42 (66.32)	241.22 (63.59)
Bottlenose dolphin	224.18 (22.68)	130.37 (28.83)	297.72 (92.22)	244.43 (22.68)
Spotted dolphin	266.97 (70.06)	266.97 (15.96)	177.01 (9.24)	375.05 (70.06)
Spinner dolphin	196.62 (33.76)	103.98 (18.82)	255.47 (85.48)	230.40 (33.76)

Table 2. Melanocyte diameter (nm²) of mysticete and odontocete species. Values shown are mean and SD.

Mysticetes			Odontocetes	Odontocetes		
240.59 (25.48)			197.58 (44.32)	197.58 (44.32)		
Bryde's whale	Fin whale	Humpback whale	Bottlenose dolphin	Spotted dolphin	Spinner dolphin	
234.07 (NA)	260.25 (26.59)	223.11 (13.46)	147.30 (8.13)	247.24 (9.94)	198.21 (14.21)	

suggesting that whales can suffer skin damage through sustained exposure to UV.¹¹

Unlike terrestrial mammals such as humans, cattle and dogs, in which melanin does not reach beyond the stratum spinosum, 26.29 cetacean melanin was distributed throughout the epidermis and present in the stratum corneum. A melanized stratum corneum would confer photoprotection to the lower epidermal layers, thus avoiding lesions associated with UV exposure, including basal cell carcinoma, squamous cell carcinoma and melanoma.³⁵ In contrast to the increasing incidence of skin cancer and actinic damage in humans, 23,28 and some other aquatic and terrestrial animals, 5,6,8,9,36-38 no form of UV-induced skin cancer has been reported to date in mysticete and odontocete cetaceans. Evidence of photodamage to the mitochondrial DNA has been detected in the skin of three species of whales^{10,30} in the absence of any evidence of oncogenic transformation.¹¹ It is feasible that a fully melanized epidermis reduces the risk of developing skin cancer, despite repeated exposure to UV. If so, this could be a unique trait of cetaceans that provides protection against damage caused by exposure to UV.

The large number of melanophages and cluster-like arrangement of melanophages observed in the skin biopsy tissue from the humpback whale was unexpected. This finding contrasts with humans where melanophages are scarce and appear as large solitary cells.³⁹ Melanophage proliferation can be associated with chronic or transient UV-associated inflammation, where they act as antigen-presenting cells for T lymphocytes.40,41 No differences in the numbers of epidermal lymphocytes were detected between species in this study. Melanophages can also increase in number in association with exposure to heavy metals and this scenario could explain the finding, although we did not detect increased numbers in the Bryde's whale and dolphin species evaluated, all of which occupy a high trophic level.⁴² Heavy metals do vary across locations and it is conceivable that the humpback whales in our study were feeding in areas where concentrations of heavy metal pollutants were increased.43 Melanophages can proliferate similarly in association with primary malignant melanomas and can be associated with cancer regression.44 However, we found no evidence of

melanoma or any other type of malignant transformation in any of the skin biopsies examined.

In this light, the large numbers of melanophages detected in most of the species evaluated in the present study suggest that they are a normal component of the epidermis and may form part of a unique photoprotective strategy of cetaceans, distinct from that described for terrestrial mammals.^{24,25,28} Cetacean evolution took place during the early Eocene.²⁰ During the Tertiary period, massive volcanic emissions of CO₂ into the atmosphere led to a reduction of the ozone layer, which resulted in increased levels of UV reaching the Earth's surface.⁴⁵ As the appearance of cetaceans and their diversification occurred during a period of high UV levels, it is plausible that this scenario caused development of more effective methods to counteract UV-induced damage. Because greater numbers of melanophages were detected in humpback whales compared to the other cetacean species, it is feasible that an unidentified epidermal stressor induces melanophage activity. This hypothetical stressor could be linked to seasonal and regional acclimatization, as humpback whales were the only species in our study with a marked migratory behaviour.^{43,46} Moreover, their melanophages were detected as large clumps, suggesting strong adhesion between these phagocytic cells and, plausibly, a reactive response dependent on cellular aggregation. A third explanation could be aging: in humans, senescence leads to an increased presence of melanophages.⁴⁷ We cannot exclude the possibility that the humpback whales included in this study were older than the rest of the individuals sampled although we did not have any means of determining the age of the individuals sampled.

In conclusion, ultraviolet radiation is considered a serious threat to both human and marine organisms because it can affect health and survival.^{1–3} In this light, it is relevant to increase our understanding of photoprotection, particularly of vulnerable wild species. This study provided basic histological and cytological information regarding the mysticete and odontocete epidermis. Future studies could utilize these data to further elucidate the protective role of the epidermis and its related structures, and to examine the evolutionary history of cetacean photoprotection.

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References

- Dahms HU, Lee JS. UV radiation in marine ectotherms: molecular effects and responses. *Aquat Toxicol* 2010; 97: 3– 14.
- Llabrés M, Agustí S, Fernandez M *et al.* Impact of elevated UVB radiation on marine biota: a meta-analysis. *Glob Ecol Biogeogr* 2013; 22: 131–144.
- Vincent WF, Roy S. Solar ultraviolet-B radiation and aquatic primary production: damage, protection and recovery. *Environ Rev* 1993; 1: 1–12.
- Diffey BL. Solar ultraviolet radiation effects on biological systems. *Phys Med Biol* 1991; 36: 299–328.
- Karentz D, Bosch I. Influence of ozone-related increases in ultraviolet radiation on Antarctic marine organisms. *Amer Zool* 2001; 41: 3–16.
- Blaustein AR, Kiesecker JM, Chivers DP et al. Ambient UV-B radiation causes deformities in amphibian embryos. *Proc Natl Acad Sci USA* 1997; 94: 13,735–13,737.
- Häder DP, Kumar HD, Smith RC et al. Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. *Photochem Photobiol Sci* 2007; 6: 267–285.
- Pruski AM, Nahon S, Escande M et al. Ultraviolet radiation induces structural and chromatin damage in Mediterranean seaurchin spermatozoa. *Mutat Res* 2009; 673: 67–73.
- 9. Sweet M, Kirkham N, Bendall M et al. Evidence of melanoma in wild marine fish populations. *PLoS One* 2012; 7: e41989.
- Bowman A, Martinez-Levasseur L, Acevedo-Whitehouse K et al. The simultaneous detection of mitochondrial DNA damage from sun-exposed skin of three whale species and its association with UV-induced microscopic lesions and apoptosis. *Mitochondrion* 2013; 13: 342–349.
- Martinez-Levasseur LM, Gendron D, Knell RJ et al. Acute sun damage and photoprotective response in whales. *Proc R Soc B* 2011; 278: 1,581–1,586.
- Kelly DL, Bothwell ML. Avoidance of solar ultraviolet radiation by juvenile coho salmon (*Oncorhynchus kisutch*). Can J Fish Aquat Sci 2002; 59: 474–482.
- Langhelle A, Lindell MJ, Nyström P. Effects of ultraviolet radiation on amphibian embryonic and larval development. *J Herpetol* 1999; 33: 449–456.
- Menon GK. New insights into skin structure: scratching the surface. Adv Drug Deliv Rev 2002; 54: S3–S17.
- Sokolov VE. Mammalian Skin. Berkeley, CA: University of California Press, 1982; 3–11.
- Harrison RJ. Functional Anatomy of Marine Mammals. London, UK: Academic Press, 1974; 44.
- Reeb D, Best PB, Kidson SH. Structure of the integument of Southern right whales, *Eubalaena australis. Anat Rec* 2007; 290: 596–613.
- Spearman RIC. The epidermal stratum corneum of the whale. J Anat 1972; 113: 373–381.
- Thewissen JGM, Cooper LN, George JC et al. From land to water: the origin of whales, dolphins and porpoises. *Evol Educ Outreach* 2009; 2: 272–288.
- Berta A, Sumich JL, Kovacs KM. Marine Mammals: Evolutionary Biology. San Diego, CA: Elsevier, 2006: 132–164.

- Mouton M, Botha A. Cutaneous lesions in cetaceans: an indicator of ecosystem status? In: Romero A, Keith EO, eds. *New Approaches to the Study of Marine Mammals*. Intech, 2012: 123–150. Available at: http://cdn.intechopen.com/pdfs-wm/407 69.pdf. Accessed September 15, 2016.
- Ilyas M. Climate augmentation of erythemal UV-B radiation dose damage in the tropics and global change. *Curr Sci* 2007; 93: 1,604–1,608.
- Lin JY, Fisher DE. Melanocyte biology and skin pigmentation. Postep Derm Alergol 2013; 445: 843–850.
- Rastogi RP, Richa, Kumar A et al. Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. *J Nucleic Acids* 2010; 592980.
- Amakiri SF. Melanin and dopa-positive cells in the skin of tropical cattle. Acta Anat 1979; 103: 434–444.
- Yamaguchi Y, Brenner M, Hearing VJ. The regulation of skin pigmentation. J Biol Chem 2007; 282: 27,557–27,561.
- Brenner M, Hearing VJ. The protective role of melanin against UV damage in human skin. *Photochem Photobiol* 2008; 84: 539– 549.
- Stevens A, Lowe J. Anatomía Patológica. Madrid, España: Harcourt, 2001; 475–510.
- Costin G, Hearing VJ. Human skin pigmentation: melanocytes modulate skin color in response to stress. *FASEB J* 2007; 21: 976–994.
- Martinez-Levasseur LM, Birch-Machin MA, Bowman A et al. Whales use distinct strategies to counteract solar ultraviolet radiation. *Sci Rep* 2013; 3: 2,386.
- Noren AP, Mocklin JA. Review of cetacean biopsy techniques: factors contributing to successful sample collection and physiological and behavioral impacts. *Mar Mamm Sci* 2012; 28: 154– 199.
- Barragán-Vargas C, Montano-Frías J, Rosales GA et al. Transformation of the genital epithelial tract occurs early in California sea lion development. *R Soc Open Sci* 2016; 3: 150,419.
- Barrandon Y, Green H. Cell size as a determinant of the cloneforming ability of human keratinocytes. *Proc Nat Acad Sci USA* 1980; 82: 5,390–5,394.
- Rice DW. Marine mammals of the world. Systematics and Distribution. The Society for Marine Mammals, Special Publication, 1998; 4: 77–79.
- Mancebo SE, Wang SQ. Skin cancer: role of ultraviolet radiation in carcinogenesis. *Rev Environ Health* 2014; 29: 265–273.
- Campbell GA, Gross TL, Adams R. Solar elastosis with squamous cell carcinoma in two horses. *Vet Pathol* 1987; 24: 463– 464.
- Hargis AM, Thomasse RW, Phemister RD. Chronic dermatosis and cutaneous squamous cell carcinoma in the beagle dog. *Vet Pathol* 1977; 14: 218–228.
- Knowles DP, Hargis AM. Solar elastosis associated with neoplasia in two dalmations. *Vet Pathol* 1986; 23: 512–514.
- Busam KJ, Charles C, Lee G *et al*. Morphologic features of melanocytes, pigmented keratinocytes and melanophages by in vivo confocal scanning laser microscopy. *Mod Path* 2001; 14: 862– 868.
- Weiss JS, James WD, Cooper KD. Melanophages in inflammatory skin disease demonstrate the surface phenotype of OKM5+ antigen-presenting cells and activated macrophages. J Am Acad Dermatol 1988; 19: 633–641.
- Cooper KD, Neises GR, Katz SI. Antigen-Presenting OKM5⁺ melanophages appear in human epidermis after ultraviolet radiation. *J Invest Dermatol* 1986; 86: 363–370.
- James WD, Cooper KD, Todd RF III et al. Inflammatory acquired oral hyperpigmentation: association with melanophages demonstrating phenotypic characteristics of antigen presenting cells and activated monocytes. *J Am Acad Dermatol* 1987; 16: 220– 226.
- Wagemann R, Innes S, Richard PR. Overview and regional and temporal differences of heavy metals in Arctic whales and ringed seals in the Canadian Arctic. *Sci Total Environ* 1996; 186: 41–66.

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- Handerson T, Berger A, Harigopol M et al. Melanophages reside in hypermelanotic, aberrantly glycosylated tumor areas and predict improved outcome in primary cutaneous malignant melanoma. J Cutaneous Pathol 2007; 34: 679–686.
- Willis KJ, Bennett KD, Birks JB. Variability in thermal and UV-B energy fluxes through time and their influence on plant diversity and speciation. *J Biogeogr* 2009; 36: 1,630–1,644.
- Bérubé M, Urban J, Dizon A et al. Genetic identification of a small and highly isolated population of fin whales (*Balaenoptera physalus*) in the Sea of Cortez, Mexico. *Cons Genet* 2000; 3: 183–190.
- Unver N, Freyschmidt-Paul P, Hörster S et al. Alterations in the epidermal-dermal melanin axis and factor XIIIa melanophages in senile lentigo and ageing skin. *Br J Dermatol* 2006; 155: 119–128.

Résumé

Contexte – Les mécanismes cellulaires utilisés pour contrer ou minimiser les dommages liés aux rayons solaires ultraviolets chez les vertébrés marins sont mal compris. Les cétacés sont vulnérables puisqu'ils n'ont pas d'annexes cutanés protectrices et sont obligés de faire surface continuellement pour respirer, s'exposant de façon répétée aux UV. Bien que les mécanismes de photoprotection moléculaire des cétacées aient été étudiés, il n'existe que des connaissances limitées sur leur structure épidermique et les effecteurs photo-protecteurs.

Objectifs – Décrire et comparer l'épiderme des cétacées mysticètes et odontocètes et identifier des facteurs potentiellement photo-protecteurs.

Sujets – Vingt-huit individus sauvages appartenant à six espèces de cétacées ont été prélevés dans le pacifique du Mexique Central et dans le golf de Californie. Les espèces prélevées étaient le Grand dauphin, dauphin tacheté pantropical, dauphin à long bec, rorqual de Bryde, rorqual commun et baleine à bosse.

Méthodes – Evaluation cytologique et histologique des biopsies cutanées prélevées entre 2014 et 2016. **Résultats** – Les cétacées avaient tous seulement trois couches épidermiques et manquaient de *stratum* granulosum et de *stratum lucidum*. Un *stratum corneum* relativement épais avec une morphologie parakeratose-like a été notée. La mélanine a été observée au sein des kératinocytes dans toutes les couches de l'épiderme, y compris le *stratum corneum* et les mélanosomes apicaux recouvrant les noyaux des kératinocytes. Les kératinocytes avaient un halo périnucléaire. Le diamètre des kératinocytes différait entre les sous-ordres des cétacés et parmi les espèces. Les amas de mélanophages étaient fréquents pour la plupart des espèces de cétacés.

Conclusions – La présence répandue de mélanine et le nombre élevé et inattendu de mélanophages pourrait constituer un caractère photoprotecteur unique des cétacés et pourrait refléter des adaptations primitives à leur environnement et à leur mode de vie marin.

Resumen

Introducción – Los mecanismos celulares utilizados para contrarrestar o limitar el daño causado por la exposición de los vertebrados marinos a la radiación solar ultravioleta (UV) son poco conocidos. Los cetáceos son vulnerables ya que carecen de apéndices protectores de la piel y están obligados a salir a la superficie a respirar de forma continua, por lo tanto, están expuestos repetidamente a la luz UV. Aunque se han estudiado los mecanismos moleculares de la fotoprotección de los cetáceos, existe un conocimiento limitado sobre su estructura epidérmica y efectores fotoprotectores.

Objetivo – Describir y comparar la epidermis de misticetos y odontocetos cetáceos e identificar rasgos potencialmente fotoprotectores.

Animales – Se tomaron muestras de veintiocho animales salvajes pertenecientes a seis especies de cetáceos en el Pacífico Central de México y el Golfo de California. Las especies muestreadas fueron el delfín mular, delfín moteado pantropical, delfín girador, ballena de Bryde, rorcual común y la ballena jorobada.

Métodos – La evaluación histológica y citológica de biopsias de piel recogidos en el campo entre 2014 y 2016.

Resultados – Todos los cetáceos tenían sólo tres capas de la epidermis, que carece tanto del estrato granuloso como del estrato lúcido. Se observó una capa córnea relativamente gruesa con una morfología similar a paraqueratosis. La melanina se observó dentro de los queratinocitos en todas las capas de la epidermis, incluyendo el estrato córneo y los melanosomas apicales ocultaron el núcleo de los queratinocitos. Los queratinocitos tenían un halo perinuclear. El diámetro de los queratinocitos difirió entre subórdenes de cetáceos y entre especies. Agregados de melanofagos fueron comunes en la mayor parte de las especies de cetáceos.

Conclusiones – La presencia generalizada de melanina y el inesperado alto número de melanófagos pueden constituir un rasgo único fotoprotector de los cetáceos y podrían reflejar adaptaciones primitivas a su entorno y su vida marina obligada.

Zusammenfassung

Hintergrund – Die zellulären Mechanismen, die bei Meerestieren eingesetzt werden, um dem Schaden durch eine Exposition von ultravioletter (UV) Sonnen Strahlung entgegenzuwirken oder ihn zu limitieren, sind noch wenig bekannt. Waltiere sind verletzlich, da sie keine Hautanhänge haben und daher immer

wieder zum Atmen an die Oberfläche müssen, wobei sie immer wieder UV Licht ausgesetzt sind. Obwohl molekulare Mechanismen der Photoprotektion bei Waltieren untersucht worden sind, besteht nur limitiertes Wissen über die epidermale Struktur und die photoprotektiven Erfolgsorgane.

Ziele – Eine Beschreibung und ein Vergleich der Epidermis von Bartenwalen und Zahnwalen und eine Identifizierung möglicher photoprotektiver Anlagen.

Tiere – Von achtundzwanzig freilebenden Individuen, die zu sechs Zahnwalspezies gehörten, wurden Proben im mexikanischen Zentralpazifik und im Golf von Kalifornien genommen.

Methoden – Die histologische und zytologische Evaluierung von Hautbiopsieproben, die im Feld zwischen 2014 und 2016 genommen worden waren.

Ergebnisse – Alle Zahnwale hatten nur drei epidermale Schichten, wobei ihnen sowohl das Stratum granulosum wie auch das Stratum lucidum fehlte. Ein relativ dickes Stratum corneum mit einer Parakeratose-ä hnlichen Morphologie wurde festgestellt. Melanin wurde innerhalb der Keratinozyten in allen epidermalen Schichten gefunden, so auch im Stratum corneum und in den apikalen Melanosomen, die den Kern der Keratinozyten verdeckten. Die Keratinozyten hatten einen perinukleären Halo. Der Durchmesser der Keratinozyten unterschied sich zwischen den Unterordnungen der Zahnwale und den anderen Spezies. Melanophagenansammlungen traten bei allen Zahnwalspezies häufig auf.

Schlussfolgerungen – Das ausgedehnte Vorkommen von Melanin und die unerwartet hohe Anzahl an Melanophagen könnte eine einzigartige photoprotektive Anlage der Zahnwale darstellen und könnte eine primitive Adaptierung an ihre Umwelt und an ihr obligates Meerwasser-gebundenes Leben sein.

要約

背景 – 海洋脊椎動物の太陽紫外線(UV)暴露による損傷を中和する、あるいは、制限する細胞メカニズムはよく分かっていない。クジラ目の生物は皮膚の保護付属器官を欠くために脆弱で、呼吸のために絶え間なく水面にいなくてはならず、繰り返し紫外光に暴露される。クジラ目の生物の光防御における分子メカニズムはこれまで、研究されてきているが、表皮構造や光防御効果器に対する情報は限られている。

目的 - クジラ目のヒゲクジラ亜目とハクジラ亜目の表皮の特徴を記載および比較し、潜在的な光防御特性を同定する こと。

供与動物 - メキシコ中部太平洋とカリフォルニア湾において、6種のクジラ目に属する28頭の自由生活性個体より採材した。採材した個体は、バンドウイルカ、マダライルカ、ハシナガイルカ、ニタリクジラ、スナガイルカ、およびザトウクジラであった。

方法 - 2014年から2016年にかけての野外調査で、採材した皮膚生検組織を、病理組織学的および細胞学的に評価した。

結果 – すべてのクジラ目が3層の表皮しか持っておらず、顆粒層および透明層を共に欠いていた。錯角化様の形態を 伴う比較的肥厚した角質層を認めた。メラニン顆粒は、角質層を含むすべての表皮層のケラチノサイト内に認められ、 頂端側メラノソームはケラチノサイトの核を不明瞭にしていた。ケラチノサイトは核周囲にハローを有していた。ケラチノサイト の直径はクジラ亜目ごとおよび種内で、異なっていた。メラノファージの集塊が、大部分のクジラ目に一般的に認められ た。

結論 ー 広範囲に分布するメラニンと予想以上に高頻度に認められたメラノファージは、クジラ目に特有な光防御特性の構成要素となっていると考えられ、クジラ目の環境や海洋に制限された生活に対する原始的な適応力を反映していると考えられた。

摘要

背景 — 对于阻挡和限制日光紫外线(UV)辐射带来的伤害,海洋脊椎动物细胞机制的相关研究很少。鲸目动物 因缺乏防护性皮肤附件更容易受伤,并且需要连续不断地到海面呼吸,因此会经常暴露于UV之下。尽管鲸目 动物光保护作用的分子机制已有研究,但对表皮结构和光保护作用感受器所知有限。

目的一描述和对比须鲸目和齿鲸目表皮,并确定其可能的光保护特性。

动物 — 采集的动物样本,是自由生活在墨西哥太平洋中部和加利福尼亚海湾的二十八只鲸目,其中囊括六个 品种。宽吻海豚、泛热带斑点海豚、长吻原海豚布莱德鲸、长须鲸、座头鲸。

方法一收集2014年至2016年该海域实验动物的皮肤活检样本,并进行组织学和细胞学评估。

结果 — 所有鲸目动物只有三层表皮层,同时缺乏颗粒层和透明层。相对较厚的角质层呈现角化不全样形态学 表现。各表皮层的角质细胞均有黑色素分布。角质细胞有细胞核周光环。鲸目各亚目和其中的品种角质细 胞直径各有不同。噬黑色素细胞聚集常见于大部分鲸目品种。

总结 — 广泛分布的黑色素,以及出人意料的大量噬黑色素细胞,可能形成了鲸目动物独特的光保护特性,也反映了对其环境和特殊海洋生活方式的原始适应性。

Resumo

Contexto – Os mecanismos celulares utilizados para neutralizar ou limitar o dano causado pela exposição à radiação solar ultravioleta em vertebrados marinhos é pouco compreendida. Cetáceos são vulneráveis já que possuem menos anexos cutâneos protetores e, obrigatoriamente, devem subir à superfície continuamente para respirar, ficando assim expostos à radiação UV constantemente. Apesar de os mecanismos moleculares de proteção solar de cetáceos já terem sido estudados, o conhecimento sobre sua estrutura epidérmica e fatores fotoprotetores ainda é limitado.

Objetivo – Descrever e comparar a epiderme de cetáceos misticetos e odontocetos e identificar características fotoprotetoras.

Animais – Foram coletadas amostras de vinte e oito indivíduos de vida livre pertencentes a seis espécies cetáceas nas regiões do Pacífico Central Mexicano e no Golfo da Califórnia. As espécies amostradas foram o golfinho nariz de garrafa, golfinho pintado pantropical, golfinho rotador, baleia-de-bryde, baleia-fin e jubarte.

Métodos - Avaliação histológica e citológica da pele coletada por biópsias, entre 2014 e 2016.

Resultados – Todos os cetáceos possuíam apenas três camadas epidérmicas, estando ausentes o estrato granuloso e o estrato lúcido. Um estrato córneo relativamente espesso apresentando morfologia semelhante à paraqueratose. Melanina foi observada entre os queratinócitos em todas as camadas da epiderme, incluindo o estrato córneo e melanossomos apicais encobriram o núcleo dos queratinócitos. Um halo perinuclear também foi observado nos queratinócitos. O diâmetro dos queratinócitos diferiu entre as subordens e espécies de cetáceos. Grumos de melanófagos foram comuns na maioria das espécies cetáceas

Conclusões – A presença extensa de melanina e o alto número inesperado de melanófagos provavelmente constituem a única característica fotoprotetora de cetáceos e pode refletir adaptações primitivas ao ambiente e à vida marinha obrigatória.