ISSN 0012-4966, Doklady Biological Sciences, 2016, Vol. 471, pp. 261–265. © Pleiades Publishing, Ltd., 2016.
Original Russian Text © O.F. Chernova, O.V. Shpak, A.B. Kiladze, V.S. Azarova, V.V. Rozhnov, 2016, published in Doklady Akademii Nauk, 2016, Vol. 471, No. 2, pp. 245–249.

GENERAL BIOLOGY

Summer Molting of Bowhead Whales *Balaena mysticetus* Linnaeus, 1758, of the Okhotsk Sea Population

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Received June 9, 2016

Abstract—In bowhead whales summering in Ulbanskiy Bay of the Okhotsk Sea, molting of epidermis has been found and histologically confirmed. The outer layer of the molting whale epidermis is longitudinally stratified and rejected in the form of relatively large plates up to several millimeters thick, each representing a lamellar formation consisting of longitudinal rows of parakeratocytes with degenerated nuclei, numerous pigment granules, and lipid inclusions. Molting intensity is correlated with the level of proliferation and regeneration of all epidermal layers, which helps to maintain the optimal skin thickness.

DOI: 10.1134/S0012496616060028

The bowhead whale *Balaena mysticetus* Linnaeus, 1758 (BW), is a typical arctic animal living near the ice edge [1]. The southernmost BW population of the Okhotsk Sea is isolated from the large population of the Bering-Chukchi-Beaufort Sea and, presumably, stays year-round in the Okhotsk Sea basin [2, 3]. The Okhotsk Sea BWs had been almost extirpated during whaling in the 19th and 20th centuries, and now they are included in the Red List of threatened species (*Red Data Book of the Russian Federation*) under category "Endangered" [4]. In summer, a significant part of this population is concentrated in the western area of the Okhotsk Sea, in the the Shantar Archipelago region [2], where, according to our observations [5–7], the whales are gathered within shallow bay areas under the conditions of temperate climate, which is not a typical environment for this Arctic species.

In cetaceans living under the conditions of indistinct climatic seasonality, growth of the epidermis layers, shedding of cellular debris, and cell replacement occur permanently at a constant rate, which provides a relatively unchangeable thickness of epidermis [8–12]. Real molting with stratification and rejection of large epidermal surface layers was considered so far a extraordinary phenomenon for whales, because it has first been described only in the beluga whale *Delphinapterus leucas* [13]. Later, the age-related and seasonal moltings were also found to be characteristic of the southern right whale *Eubalaena australis* [12]. In BW, only the age molting has been described in detail [10], although the micro- and ultrastructure of their non-molting skin is well studied [10–12, 14]. Nevertheless, marine whalers and some researchers were witnesses to BWs losting their surface plates of "skin," but these testimonies were regarded as unreliable [10]. However, according to our observations of the BW population in Ulbanskiy Bay (western part of the Okhotsk Sea), detachment of rather large epidermal plates, from thin translucent films to loose plates several millimeters in thickness, occurred in most whales of various ages in summer. This process was especially pronounced in tail-slapping or breaching whales. Earlier we regarded this phenomenon as proper molting [5, 7], but histological analysis was required for validation.

In this study, we aimed at confirming BW molting by histological methods and discussing the reasons for this phenomenon.

Samples of BW epidermis were collected in Ulbanskiy Bay in July–August 2011–2012. Tissue biopsy sampling was conducted climatic using an Excalibur Apex crossbow (Excalibur Crossbow Inc., Canada), and epidermal plates/films were collected with a fishing scoop net immediately after the whale's breaching or tail slapping (10–20 s after plate rejection). The material collected was frozen and kept at –18°C. In total, we have examined five samples of plates in the form of translucent black–grey films that were picked up in water, three samples of the epidermal debris shed from the films, four samples of the skin biopsy from four adults without any signs of molting, three samples of the loose skin biopsy from three adults having the signs of molting. The defrosted skin samples underwent fixation in 10% formalin. Preparations were made by standard techniques using the semiautomatic

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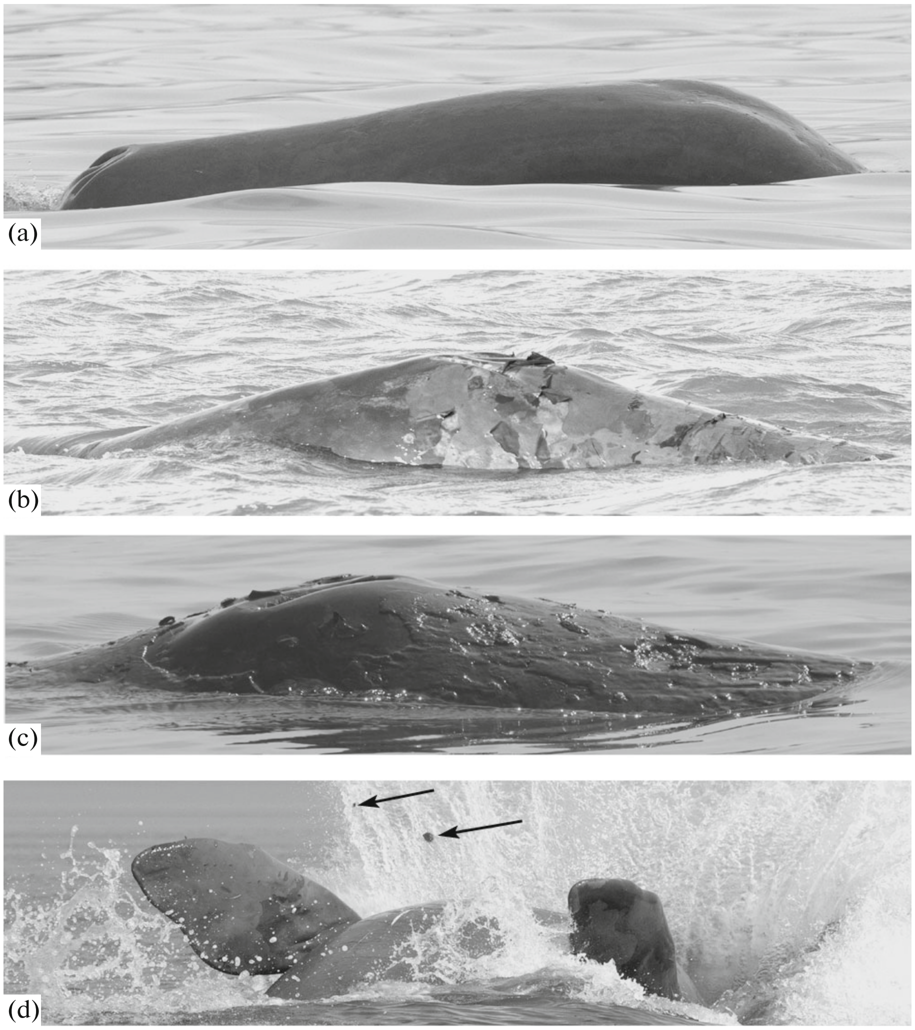

Fig. 1. Bowhead whales with non-molting skin (a) and in the state of molting (b, c, d). Arrows indicate the rejected surface layer plates, flying away when the whale is hitting the water after a breach (the ventral side up). Photo by O. V. Shpak.

histological equipment from Medite (Germany). Paraffin sections (8–13-μm thick) stained with Ehrlich's hematoxylin and eosin were embedded into LEICA CV MOUNT liquid glass (Leica Biosystems, Germany) and examined using a Keyence Biorevo BZ-9000 motorized microscope (Keyence, Japan). Morphometric data were processed statistically using the Statistica 10.0 software (StatSoft, United States).

The BW skin is mostly black or dark-grey with a white color on the chin and sometimes on the belly, tail stem, lobes, and in armpits. Some of the plates picked up from water have white star-shaped inclusions, which is indicative of their shedding from these very areas, e.g., from the tail when slapping. BW skin is usually smooth (Fig. 1a), but in many individuals, there were external signs of molting, especially pronounced on the head (Figs. 1b, 1c, 1d), where the loose skin surface is ragged, lamellar, and the skin color is often changed.

The BW epidermis is a skin layer with papillomatous (connection via long papillae between the epidermis and dermis) and parakeratose (with incomplete keratinization) structure typical for cetaceans [11, 13–15]. This layer is formed by squamous epithelium and differentiated into the stratum basale, st. intermedium, and st. externum. According to our data on four samples of non-molting skin, the average thickness of the biopsy-captured epidermal layer was 3.03 ± 0.04 mm (herein, $M \pm m$, the number of measurements on different preparations $n = 10$), and that of the corneous layer was 250 ± 50 μm ($n = 8$). Note a significant range of this parameter, from 80 to 480 μm. Thus, we had at our disposal about 1/2–1/4 of the biopsy-captured upper epidermis, where st. externum and the upper part of st. intermedium were completely represented (Fig. 2a). St. externum consists of flattened parakeratocytes with either preserved oval nuclei, slightly elongated along the skin surface, or degenerated nuclei surrounded with pigment granules

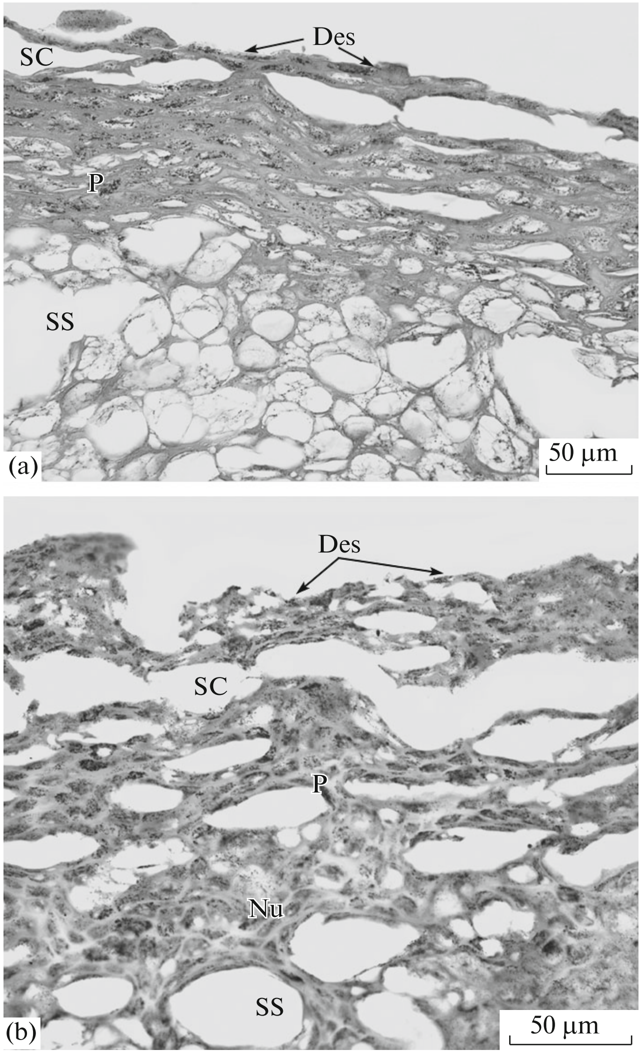

Fig. 2. The surface-layer microstructure of the non-molting skin epidermis in an adult bowhead whale: (a) general view; (b) surface parts. Des, shedding, desquamation, SC, st. externum; SS, surface area of st. intermedium; P, pigment granules; Nu, parakeratocyte nucleus. Here and in Fig. 3, microphotographs are presented; hematoxylin–eosin staining.

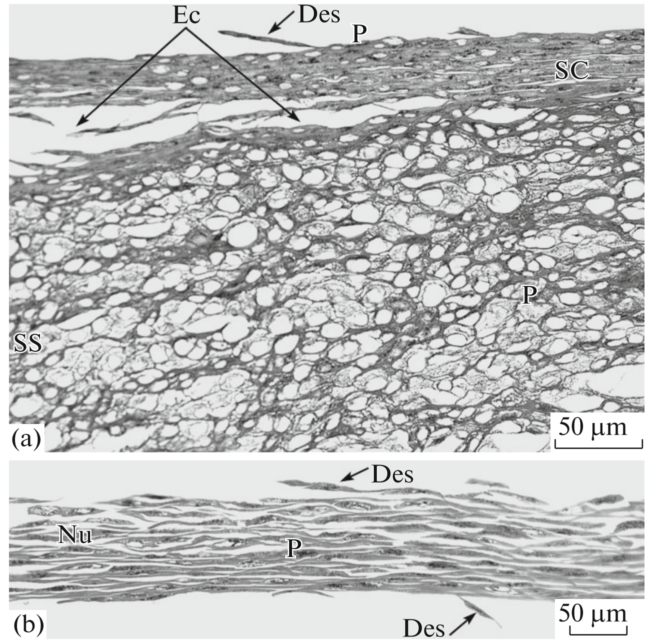

Fig. 3. Molting (stratification and rejection) of epidermis of an adult bowhead whale: (a) detaching of a plate of stratum externum (indicated with arrow); (b) microstructure of the detached plate after picking up from water. Designations are the same as in Fig. 2. Ec, molting, ecdysis.

(Fig. 2b). The parakeratocyte compactness was variable: cell layers were sometimes closely adjacent without any gaps, or on the contrary, voids between cells were clearly distinguishable (before histological degreasing they were filled with fat). There were rejected cell groups or cell debris on the surface (Fig. 2b).

The molting process, i.e., shedding and rejection of both thin and thick plates of st. externum by the way of stratification, is undoubted (Fig. 3a). These plates have a lamellar structure formed by packed flattened parakeratocytes. The densities of their packing and the degrees of stratification were different (Fig. 3b). The thickness of the three plates studied varied within a range of 110–240 μm (150 ± 20, $n = 10$). The average thickness of st. externum during stratification of the molting skin (Fig. 3a) reached 120 ± 10 μm ($n = 10$), while the thickness of the rejected plate could reach 60% of the entire layer. The number of longitudinal parakeratocyte rows in the rejected plates varied within a range of 6–15, averaging about 10.20 ± 1.07 ($n = 10$). Note that, along with stratification of st. externum, the normal process of desquamation of small parakeratocyte groups took place on its surface (Figs. 3a, 3b).

The intensity of molting was estimated by comparing the average number of the longitudinal parakeratocyte rows in the stratified epidermis with the range of this parameter in the non-molting skin:

$$K_D = \frac{\overline{k}_{SC}^{moult}}{R_{SC}} \cdot 100 = \frac{\overline{k}_{SC}^{moult}}{k_{SC}^{\max} - k_{SC}^{\min}} \cdot 100,$$

where K_D is the coefficient of molting, %; $\overline{k}_{SC}^{moult}$ is the average number of the longitudinal parakeratocyte rows in the molting skin (10 ± 0.55), R_{SC} is the a range of this parameter in the non-molting skin, $k_{SC}^{\max}$ and $k_{SC}^{\min}$ are the maximal and minimal numbers of the longitudinal parakeratocyte rows (60 and 12, respectively [10]) in the non-molting skin.

Since the rates of molting and proliferation are strictly coupled in the epidermal system to provide replenishing of a loss of parakeratocytes, then, at the

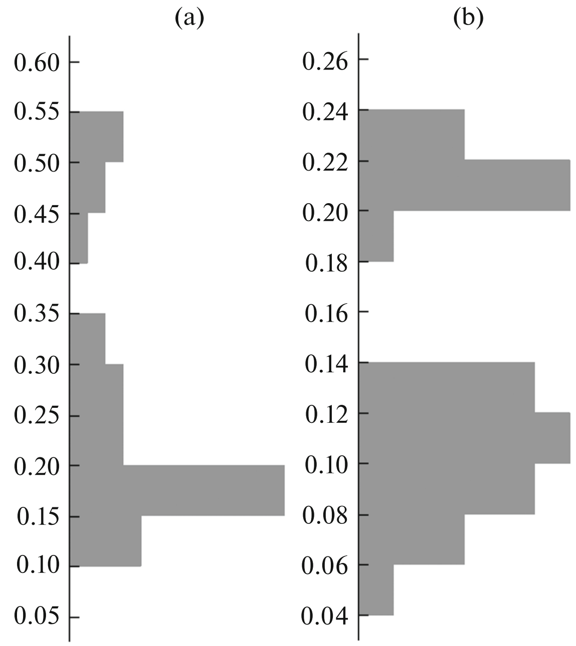

Fig. 4. Bimodal distribution of (a) the stratum externum thickness (in millimeters) and (b) the rejected plate thickness ($n = 30$) in the bowhead whale epidermis during summer molting..

calculated molting coefficient of 20.8%, the coefficient of proliferation is 79.2%. These values indicate that the proliferation intensity exceeds that of the molting, which provides the basis for BW skin homeostasis. The process of molting can be also estimated when the thickness of st. externun in the non-molting skin is compared with the thickness of the rejected plates in the molting skin, because distribution of st. externum thickness is similar to that of the number of parakeratocyte rows in the rejected plates. This dependence is indicative of a relationship between the meristic (the number of parakeratocyte rows) and plastic (st. externum thickness) features (table). Bimodal distribution was characteristic not only for st. externum of the non-molting skin, but also for the rejected plates of this layer (Figs. 4a, 4b).

Thus, visual observations of BW and histological validation: indicate in summer, in BW of the Okhotsk Sea population along with permanent skin shedding, seasonal molting, i.e., an intense abruption of st. externum surface layers, is characteristic of these animals. St. externum of the molting BW is longitudinally stratified and rejected in the form of thin or thick (up to several millimeters), rather large plates that have lamellar structures consisting of longitudinal rows of parakeratocytes. Molting intensity is considerably high, but it does not exceed the intensity of proliferation, which helps to maintain skin homeostasis. We believe that molting is related to BW's moving into the well-warmed shallow bays of the western Okhotsk Sea and has an adaptive significance, i.e., serves for cooling of the outer BW integument and improving its metabolism, water resistance, strength and damping properties, as well as leads to a partial loss of parasites.

We also believe that skin molting is characteristic of the whales, which migrate between the marine areas with different temperature and salt conditions, to a greater extent than previously thought, which will be, probably, confirmed by further studying of more cetaceous species.

Thickness of st. externum in the non-molting skin and abrupted plates of the bowhead whale during summer molting (the number of measurement $n = 30$)

Sample	Thickness of st. externum, mm
	$M \pm m$
Non-molting skin	0.26 ± 0.02
Rejected plates	0.14 ± 0.01

ACKNOWLEDGMENTS

We are deeply grateful to A. Yu. Paramonov for participation in field observations and sample collection, as well as to G. I. Malyshevskiy (Gold mining Artel Vostok, Khabarovsk) for the help in organizing the field work in Ulbanskiy Bay.

The field observations and sample collection were supported by the Ocean Park Corporation, Hong Kong, and Georgia Aquarium Inc., United States (the project "Current Status of the Sakhalin–Amur Beluga Aggregations"). The study has been conducted as a part of the Permanent Expedition of the Russian Academy of Sciences studing the RF Red Data Book animals and other important Russian fauna species with financial support of the Russian Geographical Society (The "Beluga, White Whale Program").

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Translated by A. Nikolaeva