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ASSESSMENT OF REPRODUCTIVE HORMONES LEVEL IN BALEENS OF BOWHEAD WHALES AND IN SERUM OF GRAY WHALES OFF CHUKOTKA (RUSSIA)

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ABSTRACT

The level of reproductive hormones (testosterone and progesterone) in the serum of gray whales (six individuals) and Pacific walrus (4 individuals) harvested in Chukotka was analyzed. Immature gray whales had average level of progesterone 0.37 ± 0.04 ng/ml and testosterone 0.58 ± 0.13 ng/ml. Such low concentrations are associated with the age of whales that have not yet reached puberty. Pacific walruses had an average progesterone level of 0.97 ± 0.40 ng/ml (lim=0.47÷2.18) and testosterone 0.31 ± 0.05 ng/ml (lim=0.24÷0.46). For the first time in the Russian Federation, the level of progesterone and testosterone in the Bowhead whales was determined. The analysis made it possible to trace changes in the hormonal status of animals during first 9 years of life of two individuals. It was shown that puberty in the bowhead whale (an increase in testosterone levels) is observed at the age of about 9 years. It was noted that newborn cubs (under six months) had a very high level of testosterone, which theoretically could be connected with their exposure to maternal testosterone during the period of animal embryonic development. We will continue an extremely promising study of the hormones of whales, walruses and seals harvested in the Chukotka coastal waters with the Chukotka Autonomous Region Government support.

Key words: Bowhead and Gray whales, Pacific walrus, baleen, serum, harvest monitoring, western Bering and Chukchi Seas, testosterone, progesterone, steroid hormone, concentration

The assessment of the physiological status of mammals in the wild, including their hormonal and immune status, has increasing interest using non-invasive methods [1; 2]. Determination of hormone concentrations in wild animal excretes (excrements [1] and urine [3]), as well as in hairs [4], provide information on the stress of animals [3], the state of the reproductive system, including breeding periods [5], ovulation [6], and animal pregnancy [7]. Using of various excretes and hairs allows to consider the results on different time scales, getting information about what happened several hours ago (urine), days ago (excrements) or months ago (hairs). An important role in conducting adequate analysis is played by the preservation of hormones in the excrements, urine or hairs, which is significantly influenced by the temperature and humidity of the samples [7; 8]. Low negative air temperatures along with a dry climate are the guarantor of the samples safety.

Conducting of such studies on cetaceans is extremely difficult, animals excrete the feces and urine directly into the water, so it is almost impossible to collect samples and obtain adequate results. In some studies of live-captured cetaceans the information on the concentration of hormones in the blood has been obtained, but these data refer to small species [9]. Excrements for the analysis of hormonal status of cetaceans were used for killer whales [10]. Another approach was an attempt to estimate the level of hormones in the exhalation of different dolphins and whales [11]. Captured in captivity, this method did not yield significant results when used in the wild, mostly because of the difficulty of collecting the materials.

An alternative approach obtaining a retrospective assessment of the state of post mortem whales was the measurement of steroid hormones in the whale baleens, first performed on the Bowhead whale [12; 13]. The concentrations of cortisol and progesterone in the bowhead whale baleens were determined, and a validation was subsequently carried out, confirming the validity of such measurements. An analysis of the growth rates of the bowhead whales made it possible to estimate the time frame corresponding the measurements taken.

The Gray and Bowhead whales inhabit the northern Pacific, including the east coast of Russia, and for both the IUCN status is 'Least concerned'. The Gray and Bowhead whales are traditional target for Chukotka Natives, who use its meat for subsistence [14]. Using of parts of harvested animals and evaluation of the level of steroid hormones in their baleens can provide unique information on the stress and physiological status of marine mammals in this region.

The main goal of this research was to assess the level of steroid hormones in baleens and serum of harvested whales.

MATERIAL AND METHODS

Cetaceans (beluga whale, gray and bowhead whales) and pinnipeds are harvested by the Chukotka Natives only for subsistence and to keep their traditional way of life.

Traditionally, the largest number of gray whales is harvested off the Chukotka Peninsula coast by the whalers of the largest national Chukotka village of Lorino, therefore it was chosen in the period of 2017-2018 for harvest monitoring.

The serum and baleens' samples of whales, harvested by Native whalers under IWC allocated quota were collected and analyzed in 2003-2018. The baleen samples were stored dried before the extraction. For the extraction of the hormones two baleens were drilled (diameter 4 mm) from basal to distal side by every 4 cm, which corresponded to the sampling time after one year of growth [13]. The obtained fine particles (all pieces larger than 1 mm in diameter were removed) were weighed into a 15 ml tube with an accuracy of 0.0001 g, and 10 ml of 100% methanol per 0.1 g of baleen samples was added. The tube was placed in a Mini-Rotator BIO-RS-24 (Korea) for 24 hours at room temperature. After that 1.5 ml of liquid was taken into an Eppendorf tube and centrifuged for 10 minutes at 1300 G. At the end of the procedure, 1 ml of supernatant was taken into a clean Eppendorf tube and completely evaporated at 55°C. PBS was added to each tube in a volume of 200 µl, shaken for five minutes on vortex and then frozen at -18°C until measurements were taken.

The level of reproductive hormones was determined by the enzyme immunoassay using commercial Testosterone-Plus and Progesterone-Plus kits (Xema, Moscow, Russia), kit sensitivity were 0.016 ng/ml and 0.015 ng/ml respectively. Measurements were established on a Multiscan EX (Thermo, Finland) plate spectrophotometer.

Totally the hormone concentrations were determined in 74 (37 from unknown sex and 37 from female) samples from two Bowhead whale baleens. Unknown sex was collected from washed carcass in Nutepelmen village (Chukchi Sea) at 2017 and second baleen was from pregnant female, harvested in Lorino village (Bering Straight area) at 2016.

The statistical analysis was carried out using the Statistics 8.0 software. A comparison of the concentrations of hormones in different segments of each baleen was performed using the Wilcoxon test for conjugated pairs. A comparison of hormone concentrations in males and females was performed using the Mann-Whitney test.

RESULTS AND DISCUSSION

The average testosterone level in all gray whale samples was extremely low and was about 0.5 ng/ml (Figure 1a).

In the female it was slightly higher than the average in males; however, in individual males it was comparable to that in the females. The average level of progesterone in gray whales was also less than 1 ng/ml, including the female, in which it was 0.30 ng/ml, which ruled out the likelihood that the female was pregnant (Figure 1b).

Progesterone and testosterone concentrations in two bowhead whales analysis has shown, that in the baleen from whale found in 2017 in Nutepelmen the average testosterone concentration was 1.8 ± 0.2 and progesterone 414 ± 133 ng/g; for baleen from female harvested in 2016 respectively 1.8 ± 0.1 and progesterone 242 ± 44 ng/g. Genetic analysis was not carried out, but the data obtained from the concentrations of progesterone, and most importantly, its dynamics, suggest that we analyzed samples from one female (2016) and one male (2017).

Nevertheless the most interesting were hormonal profiles of an animal with undetermined sex (Figures 2 a,b). Considering the approximate rate of growth of the bowhead whale's signature, the level of progesterone was extremely low during the first two years, but a slight increase was observed at the age of about 7 years. Perhaps this rise in progesterone levels can be associated with the onset of puberty of the animal (Fig. 2): progesterone is a precursor in the synthesis of testosterone and other androgens (for example, androstenedione), which may explain its relatively high level during this period. The level of progesterone did not correlate significantly with the level of testosterone ($r = 0.23$), which suggests that the measurements of hormones were independent for the stored individual parts of the baleens.

Testosterone levels were less affected in gray whales. Over the years it changed only 1.5-2 times. The rise of testosterone level at the age of 9 years (103-109) (not registered in the female whale) and the relatively high level of testosterone at the age of 1-3 months stood out by themselves.

Measurements of hormone concentrations in the serum of male walruses showed that progesterone levels were slightly higher in animals than testosterone levels (Fig. 3).

The hormonal status of cetaceans has been studied poorly. Information about it is based mainly on the study of blood samples of various dolphins in captivity conditions [10]. Capturing animals in nature usually leads to an increase in stress levels of animals (which is also very differ from basal level), which can adversely affect the level of reproductive hormones. Such an assessment may rather weakly reflect the real level of hormones in animals. Of course, this problem may be even more relevant in the case of animals' harvest. However, this is often the only available approach to assessing the state of animals, so the possibility of collecting biological samples should not be missed.

Analysis of gray whale serum showed extremely low concentrations of progesterone and testosterone in the samples, which is probably due to the fact that the animals were immature. This also explains the lack of sex differences in the level of hormones - in many species they are recorded only after the onset of puberty.

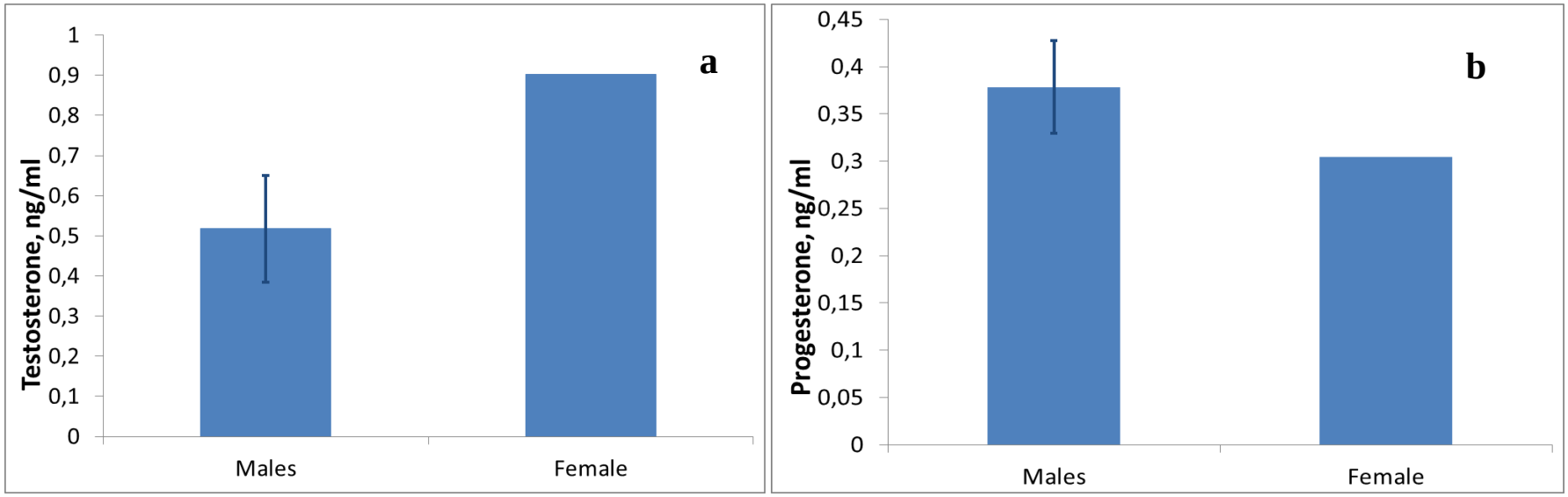

Figure 1 – Levels of testosterone (**a**) and progesterone (**b**) in serum samples from Gray whales, harvested in Chukotka (Russia) at August, 2018

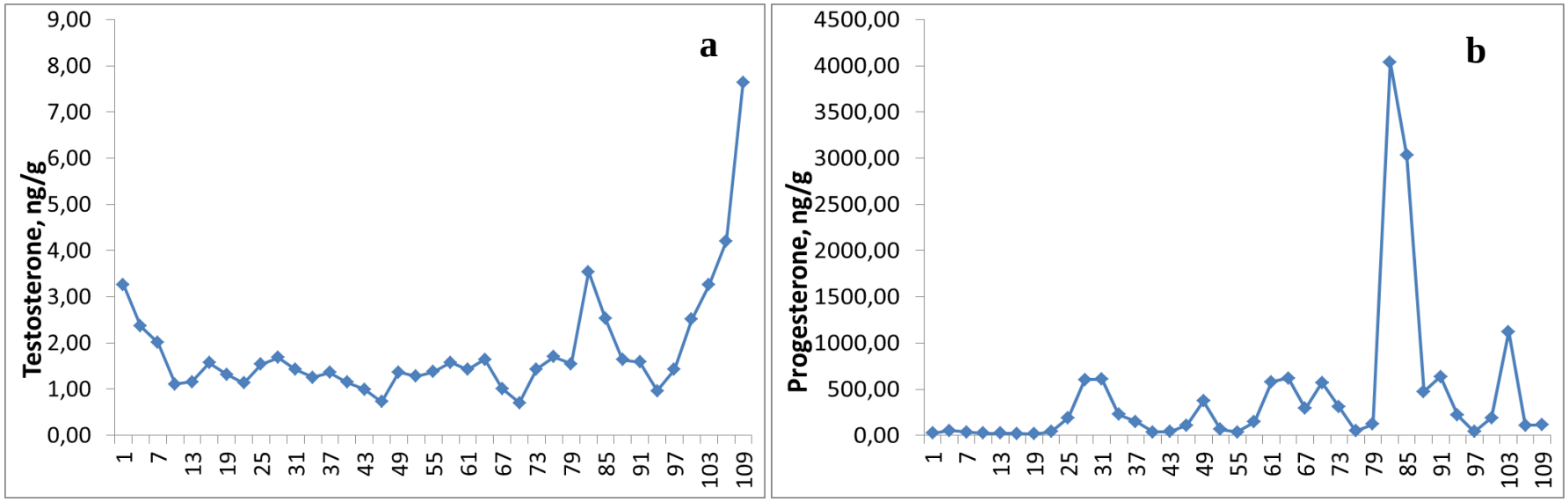

Figure 2 – Levels of testosterone (**a**) and progesterone (**b**) in baleen samples from Bowhead whale, found washed in Nutepelmen (Chukotka, Russia) at October, 2017. Lower axis is age of whales in months

Progesterone and testosterone concentrations in male walruses were also low. However, if everything is quite clear with progesterone - adult males should have extremely low concentrations of progesterone, a low level of testosterone is somewhat unexpected. Three of the four animals were sexually mature, and August is the period when males begin to seek mating with females. It is possible that such a low level can be determined by the high stress of animals during the Native harvest, which led to a dramatic decrease in testosterone levels. Analysis of the level of cortisol in serum will allow us to find the answer to this question in the future. On the other hand, absolute estimates of the testosterone level in walruses' serum are extremely difficult to compare with similar results due to the use of different antibodies. Taking blood from wild walruses or even walruses in zoos would significantly help to establish the basal level of reproductive hormones for these species. A slightly higher level of progesterone can also be a consequence of stress by harvested animal, when during synthesis of high concentrations of the cortisol a large amount of the progesterone (a predecessor of the cortisol) is synthesized.

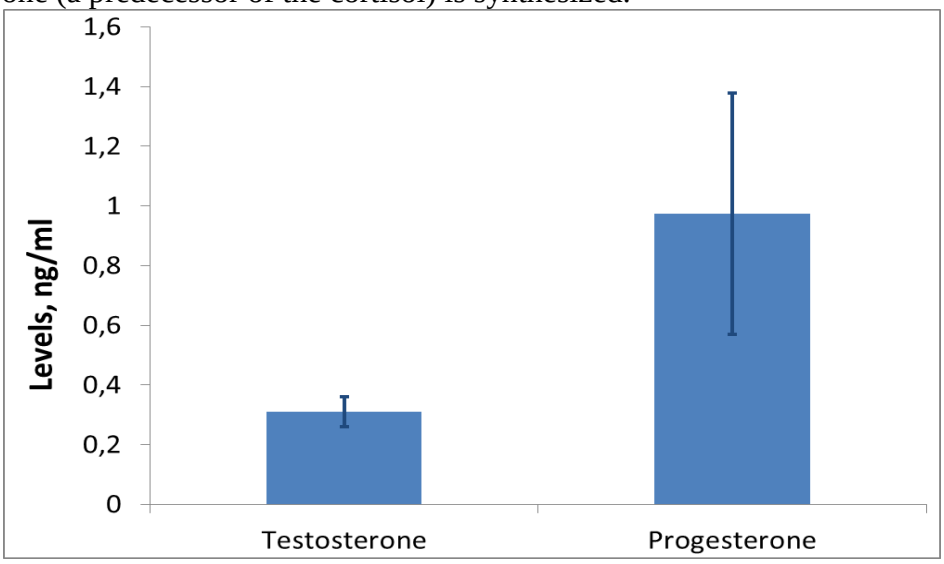

Figure 3 - Levels of testosterone and progesterone in serum samples from Pacific walruses, harvested in Chukotka (Russia) at August, 2018

Finding alternative approaches assessing the hormonal status of cetaceans in nature is extremely difficult, primarily because of their specific habitats. Almost the only study of the vital hormonal status of cetaceans in the wild remains the study of Ayres et al. [10] on killer whales, showing increased stress in the Seattle Gulf due to scarcity of foraging resources during this period.

Due to the scarcity of the data obtained in the wild, a retrospective analysis based on the analysis of the Bowhead whale baleen showed the validity of the evaluation of the steroid hormones level in animals in such way that allows us to evaluate the stress level of the animal several years ago [12; 13]. It was revealed [15, 16] that the growth rate of the whale baleen is about 16-20 cm/year, so the samples taken after 4 cm correspond to about 3-3.5 months of the animal life.

First time in Russia the analysis of testosterone and progesterone levels in the Bowhead whale baleens was conducted. Concentrations were determined and ranged for progesterone from 16 to 4040 ng/g and for testosterone from 0.72 to 7.63 ng/g. For the first time data were obtained on the level of reproductive hormones in the Bowhead whale baleens, which allow to reconstruct

changes in hormone levels during the life of the animal. This study shows a sharp increase in testosterone levels in an animal aged about 9 years, which we associate with the onset of puberty in the male. Further genetic analysis of those samples is required to confirm the sex of the animal and to study the level of stress hormone-cortisol to evaluate our interpretation of changes in the level of progesterone in the animal.

It is interesting to note that during the first months of life the level of testosterone in the animal was quite high and gradually decreased. A high testosterone level immediately after birth can be in general determined by intrauterine exposure to a high level of androgens of a pregnant female (mother), which can affect the level of hormones in the young. Together with that, in Eurasian lynxes [17], for example, this effect takes place during the first month of postembryonic development, while in whales an elevated and decreasing testosterone levels are noted for several months.

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