

# Health monitoring and the application of promoting animal welfare in captive beluga whales, *Delphinapterus leucas*

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To date, the health and welfare of all captive animals is a growing concern., however, animal welfare evaluation is a difficult task. Behavioural and physiological parameters are commonly used, but their interpretation is not always robust. Based on the above reasons, cortisol of skin measurement and microbiome analysis could become an alternative method for health and animal welfare evaluation in captive beluga whales, *Delphinapterus leucas*. Skin sampling is a relatively minimum or non-invasive sampling techniques compared with blood sampling for cortisol measurement. To evaluate the chronic stress in cetaceans, the scraped samples were collected non-invasively from three captive belugas and cortisol was extracted using chemicals to create an immiscible layer for purification, and then the levels of cortisol were detected via commercially available enzyme-linked immunosorbent assay (ELISA). Additionally, the microbial communities of the gut also influence many aspects of health in cetaceans. Reportedly, the V1–V3 region of 16S rRNA gene has been widely used as a genetic marker for bacteria. Alternatively, various studies have been targeted the 16 rRNA V3-V4 hypervariable region for the microbiome research. However, to the best of our knowledge, there was no study about the selection of appropriate 16S target amplicons in cetacean microbiota. Therefore, the resolution of V1-V3 and V3-V4 16S rRNA regions were conducted using Illumina MiSeq to estimate the microbial community diversity. The results showed that the V1-V3 revealed significantly higher species richness than the V3-V4 in fecal and blowhole samples of captive belugas. However, they did not exhibit significantly difference in specie evenness. Moreover, there is an evidence of a decrease or a ceasing in the acoustic activity of beluga in the presence of natural predators or engine noise disturbance. This response has been observed in both captive and free-ranging beluga whales and has been associated with threat, startle, fright, alarm, or stress contexts. To evaluate acoustic activities as an indicator of welfare in the belugas, the acoustic activity of three captive beluga whales was monitored during scheduled construction near to the whale pool. The result showed that number of calls on construction day has significantly decreased the acoustic activity in beluga, this activity then returned to average level after construction day. This result indicated that vocalization of beluga whale was highly correlated to environmental change and associated stress. We proposed that measuring acoustic activity in captive beluga whale would be valuable in animal welfare evaluation and management of captive cetacean.

*Keywords:* *Delphinapterus leucas*, cortisol, microbiome, acoustics, animal welfare