



HOKKAIDO UNIVERSITY

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学 位 論 文 題 名

Development of non-invasive techniques to measure testosterone in the northern fur seal (*Callorhinus ursinus*)

(キタオットセイにおけるテストステロンの非侵襲的な測定手法の開発)

The reproductive physiology of wildlife can provide useful insights into population dynamics. Information about the physiological state of animals is generally collected through blood sampling, which involves capture of animals. Non-invasive techniques of endocrine monitoring, however, have been developed to study the reproductive status of animals using indicators such as faeces and hair. The least invasive method examines faeces, since faecal collection does not require physical contact with animals. Faecal hormones are now commonly studied in captive animals. An advantage of hair sampling is that hair can be biopsied in the wild. In this study, faeces and hair were used in place of blood to study the reproductive status of the northern fur seal (*Callorhinus ursinus*) males.

The male northern fur seal becomes sexually mature at age 4, but usually becomes capable of establishing breeding territories (*i.e.*, socially mature) at age 8. Spatial segregation of males occurs during the non-breeding season off southern Hokkaido in the Sea of Japan where they have been increasingly plundering fish from fishing gear. Development of a maturity-assessment method for free-ranging seals using non-invasive techniques could enhance our understanding of males in this community. The objective of this study was to measure testosterone in male northern fur seals using non-invasive techniques and to determine the sexual maturity of free-ranging seals during the non-breeding season.

At first, the relationships between serum testosterone and faecal testosterone metabolites in a captive seal were investigated to develop non-invasive monitoring of faecal testosterone metabolite levels. And a significant relationship between the faecal testosterone metabolites and serum testosterone levels was

observed when the faeces were collected approximately one day after blood withdrawal. The seasonal changes in faecal testosterone metabolites also showed similar trends with serum testosterone levels, showing higher testosterone levels during the breeding season. These results indicate that serum testosterone levels can be estimated using faecal testosterone metabolites.

Next, the concentrations of faecal testosterone metabolites with age, sampling months and testis weight of free-ranging northern fur seals was compared to evaluate their sexual maturity. Forty-six males were collected in 2011–2017 in waters off Hokkaido. No significant differences in monthly faecal testosterone metabolites were obtained among the age class nor testis weight. These results indicate that faeces are a more useful tool in captivity since factors in the captive environment such as prey are often controlled so that faecal steroid metabolites likely are comparable in captive conditions.

Additionally, the sexual maturity of males off Hokkaido was examined using hair testosterone levels. Fifty-seven males were collected in 2011–2018. The testosterone levels in hair of juveniles were significantly lower than those of mature seals. Seals collected in May, during the spermatogenesis progresses, showed the highest testosterone levels. These results demonstrate that juvenile males can be distinguished from mature males using hair testosterone levels in May preceding the breeding season.

In conclusion, non-invasive techniques to assess the reproductive status of northern fur seal males were developed. In captivity, faecal testosterone metabolites were a useful tool to evaluate the status during the breeding season, whereas hair was an invaluable tool to determine the maturity of free-ranging seals. Data on male reproductive status can enhance our understanding of fur seal ecology, such as the distribution of the seals off Hokkaido during the non-breeding season. This method can be applied in various pinnipeds and will be especially useful for species that spend time offshore, since hair can be biopsied in the wild.

The committee members have highly evaluated these achievements. In addition, she is sincere and enthusiastic as a researcher, and have sufficient qualifications to receive a PhD. Therefore, the committee reached to a conclusion that Mayuko Otsuki deserves to become a Doctor of Environmental Science.