学　位　論　文　内　容　の　要　旨

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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 and anaesthesia of animals. In the past few decades, however, non-invasive techniques of endocrine monitoring 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. As a result, faecal hormones are now commonly studied in captive animals. An advantage of hair sampling is that hair can be biopsied in the wild. In the present study, I used faeces and hair in place of blood to study the reproductive status of the northern fur seal (*Callorhinus ursinus*) males.

The sexually dimorphic northern fur seal congregates in rookeries during the breeding season in summer, and occurs in offshore waters without hauling out during the non-breeding season in winter. Males become sexually mature at age 4, but usually become capable of establishing breeding territories (*i.e.*, socially mature) at age 8. Socially mature males return to their rookeries to establish territories before the breeding season, while juveniles remain offshore during the breeding season. 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. Since demographic information about males can help evaluate the impact of this plundering, 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.

In Chapter 2, I examined the relationships between serum testosterone and faecal testosterone metabolites in a captive seal to develop non-invasive monitoring of faecal testosterone metabolite levels. I found a significant relationship between the faecal testosterone metabolites and serum testosterone levels when the faeces were collected

approximately one day after blood withdrawal. In addition, the seasonal changes in faecal testosterone metabolites 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. This method will be a useful tool to non-invasively monitor the reproductive cycles of male northern fur seals.

In Chapter 3, I compared the concentrations of faecal testosterone metabolites with age, sampling months and testis weight of free-ranging northern fur seals to evaluate their sexual maturity. Forty-six males were collected in 2011–2017 in waters off Hokkaido. The testis weight and age of the fur seals showed a significant positive relationship although no monthly differences were obtained between mature and socially mature seals. Significant differences in monthly faecal testosterone metabolites between mature and socially mature seals were obtained only in April. Furthermore, the individual differences in the faecal testosterone metabolite levels in mature and socially mature animals were large, possibly because environmental cues such as prey availability and energetic demands can differ among individuals and differently affect endocrine systems. My results also indicate that faeces are a more useful tool in captivity since factors in the captive environment such as photoperiod and prey are often controlled so that faecal steroid metabolites likely are comparable in captive conditions.

Hair is another endocrine matrix that can assess the reproductive status of animals non-invasively. However, it is unknown if hair samples can assess pinniped reproductive status. In Chapter 4, I examined the sexual maturity of males during the non-breeding season off Hokkaido using hair testosterone levels. A total of 57 hair samples were collected from males during the non-breeding seasons of 2011–2018. The testosterone levels of juveniles were significantly lower than those of mature seals. Elongated spermatids, which occur in the final phase of spermatogenesis, were present in seals collected between April and June. Seals collected in May, during the spermatogenesis progresses, showed the highest testosterone levels. My 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. An advantage of these methodologies is that they do not disturb the animals before sampling. Furthermore, 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, and clarify the connection between non-breeding and breeding seasons. 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.