Diet of the Australian sea lion (*Neophoca cinerea*): an assessment of novel DNA-based and contemporary methods to determine prey consumption

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THESIS ABSTRACT

A fundamental prerequisite in the conservation and management of endangered species is knowledge of diet, because diet provides information on habitat use and resource requirements. However, understanding diet in marine mammals is difficult because direct feeding events are rarely observed. To overcome these limitations, many studies use the identification of skeletal remains (hard parts) recovered from faeces, or regurgitates. Yet, for the endangered Australian sea lion (*Neophoca cinerea*) (ASL), one of the rarest pinniped species in the world, diet remains a key knowledge gap that impedes our understanding of the species ecology and connectedness to other taxa in the marine ecosystem.

When this thesis commenced, knowledge of ASL diet was based on few hard part studies comprising small sample sizes, which were limited in temporal and spatial extent. Knowledge of prey utilised by ASL was poor because prey hard parts are completely digested, or, if recovered in faeces, heavily eroded. Therefore, traditional methods of dietary analysis are 'unreliable' and biased toward robust prey. However, limitations notwithstanding, the analysis of Australian sea lion diet via traditional methods still provides useful information on prey species consumed that cannot be readily obtained using other methods. For example, alternative biochemical methods, such as fatty acid and stable isotope analyses, have provided important insights into habitat use the broader trophic levels of prey consumed by ASL; however, they are yet to provide reliable taxonomic information on the diversity of prey species consumed, at least not without first having a thorough understanding of Australian sea lion prey.

Given the paucity of information on ASL diet, I initially aimed, as presented in Chapter 2, to investigate the diet of the ASL at different breeding colonies in South Australia. This initial study provided insights into some of the prey taxa consumed by ASL, which were

subsequently used to develop a range of DNA-based dietary analyses to determine consumption of different prey.

In order to apply DNA-based dietary analysis methods to wild populations, it was important to assess the application of different methods in a controlled environment to understand methodological constraints and refine the methods. In Chapter 3, I present feeding trials on captive ASL, with the aim to: i) assess end-point PCR and quantitative real-time PCR (qPCR) DNA-based techniques to determine their suitability to amplify and detect prey in ASL faeces and, ii) compare the DNA diet results with prey detected and identified using traditional hard-part methodology.

Having successfully applied faecal DNA-based methods in a controlled feeding experiment to identify different prey, I applied DNA-based methods to faecal samples collected from two ASL breeding colonies in South Australia and identified a range of prey. The aims of Chapter 4 were to: (i) determine the diversity of prey taxa by sequencing a large number of clones from a few individuals, (ii) compare the prey taxa recovered at two study sites, and (iii) determine whether pooling faecal DNA from multiple individuals provides a useful means to characterise diet at the colony/population level.

Finally, Chapter 5 utilised and extended the information gained from using the DNA-based faecal analyses presented in previous chapters, by integrating next-generation sequencing (NGS). Next-generation sequencing has the capacity to provide a greater depth of DNA sequencing than the cloning-sequencing approach, with the method potentially improving prey diversity information for the ASL. The aim of this study was to use DNA-based faecal analysis and NGS technology at one breeding colony to investigate seasonal and annual variation in prey consumed by ASL.

ACKNOWLEDGMENTS

"The very basic core of a man's living spirit is his passion for adventure. The joy of life comes from our encounters with new experiences, and there is no greater joy than having an endlessly changing horizon". McCandless

There are few opportunities as an ecologist that you get to spend time exploring the unknown. This has been particularly true for this project on the Australian sea lion (*Neophoca cinerea*); few have visited the island colonies and even fewer have had the opportunity to co-exist with the inhabitants. My PhD presented an opportune moment akin to historical exploration; though modern equipment has engaged a more comfortable existence in times of adversity, there is a certain allure that draws one back to these rugged, windswept, and captivating islands. Working on a project to assist the future conservation management of an endemic and endangered species has also been a humbling experience. The behavioural characteristics among individuals at different colonies presented many challenging moments. Some could be regarded as "lover's sunsets", others not so passionate endeavours to a point of "you take my DNA and I'll take some of yours". Battle scars aside, Australian sea lions exhibited an unusual tenacious resilient spirit, which deserves full respect and support through future conservation programs.

Many people need to be thanked for the folds of this PhD. First, I would like to thank my PhD supervisors Prof Simon Goldsworthy and Dr Kathy Ophelkeller. Simon, we have spent many years together, catching and tracking sea lions and had some of the most memorable adventures and encounters. Thank you for your ongoing support and guidance as program leader of Threatened, Protected, and Endangered Species (TEPS) at South Australian Research and Development Institute (SARDI). Ultimately, your passion of Australian sea lions and their conservation is the key reason I have been able to experience

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LIST OF PUBLICATIONS

Chapter 2

Peters, K. J., McIntosh, R. R., Shaughnessy, P. D., Baylis, A. M. M. and Goldsworthy S. D. Diet diversity and estimates of prey size of the endangered Australian sea lion (*Neophoca cinerea*) in South Australia. *In review.*

Chapter 3

Peters, K. J., Ophelkeller, K., Bott, N. J., Herdina, H., and S. D. Goldsworthy. (2014). PCR-based techniques to determine diet of the endangered Australian sea lion (*Neophoca cinerea*): a comparison with morphological analysis. *Marine Ecology,* **36** (4), 1428 – 1439. doi: 10.1111/maec.12242

Chapter 4

Peters, K. J., Ophelkeller, K., Bott, N. J., Deagle, B. E., Jarman, S. J., and S. D. Goldsworthy (2014). Fine-scale diet of the Australian sea lion (*Neophoca cinerea*) using DNA-based analysis of faeces. *Marine Ecology,* **36** (3), 1–21. doi: 10.1111/maec.12145.

Chapter 5

Peters, K. J., Ophelkeller, K., Bott, N. J., Deagle, B. E., Jarman, S. J., and S. D. Goldsworthy. Insights into seasonal prey use by the Australian sea lion (*Neophoca cinerea*) using faecal DNA and high-throughput sequencing. *In review.*

Additional publication containing results from thesis

Goldsworthy, S. D., Page, B., Rogers, P. J., Bulmand, C., Wiebkin, A., McLeay, L. J., Einoder, L., Baylis, A. M. M., Braley, M., Caines, R., Dalye, K., Huveneers, C., Peters, K., Lowther, A. D., Ward, T. M. (2013). Trophodynamics of the eastern Great Australian Bight ecosystem: Ecological change associated with the growth of Australia's largest fishery. *Ecological Modelling*, **255**, 38 –57.