Australian Marine Mammal Centre

Final Report

(subclause 9 and Schedule Item 5 of the Funding Agreement)

- **Project No**. – 0708/28
- **Title** - Population-level dietary genotyping: a re-evaluation of Australian fur seal diet by pyrosequencing of prey DNA in faeces
- **Chief Investigator** – Dr Bruce Deagle
- **Organisation** – Dr Bruce Deagle

Activity Period – 27 November 2007 to 13 December 2008

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1. Activity Summary

A clear summary of approximately 500 words outlining the work undertaken and any significant findings (for publication on the Department's web site)

The aim of this project was to re-evaluate the diet of the Australian fur-seal (*Arctocephalus pusillus doriferus*) by identifying prey DNA in faeces using recently developed high-throughput DNA sequencing technology. The data provide a unique view of Australian fur-seal diet since they are independent of prey hard-part recovery. The methololgy developed also provides a template for future DNA-based diet studies on marine mammals.

During the study, faecal samples were collected at three breeding colonies across the seals' range (Lady Julia Percy, Seal Rocks and The Skerries). DNA was extracted from 270 samples and four gene regions were amplified using PCR. A blocking primer was used to limit amplification of fur seal DNA. The amplified DNA markers from each colony were sequenced using the Roche GS-FLX platform, generating >20,000 DNA sequences. To aid in identification of sequences obtained from the fur seal faecal samples, mtDNA 16S sequences were obtained from voucher specimens of 48 fish species found in the Bass Strait region. Software was also developed to sort and group the large number of sequences produced during the current project.

A total of 54 bony fish, 4 cartilaginous fish and 4 cephalopods were identified in the faecal samples based on the most taxonomically informative markers sequenced (mitochondrial 16S). Four fish species accounted for 72% of the mtDNA sequences; confirmed as key prey of the seals were redbait (33%, *Emmelichthys nitidus*), jack mackerel (23%, *Trachurus declivis*) and barracouta (9%, *Thyrsites atun*). Blue mackerel (7%, *Scomber australasicus*) may be more important diet item than previously recognised, accounting for 25% of the sequences recovered from faeces collected at The Skerries . The relative importance of more economically valuable species does not appear higher than hard-part analysis has indicated. Barracouta and flathead species are verified as being important secondary prey, while key economic species, such as snapper (*Chrysophrys auratus*), warehou (*Seriolella* spp.), trevally (*Pseudocaranx* spp.)

and morwong (Family Cheilodactylidae), appear to be only occasional prey. One possible exception is Pink Ling (*Genypterus blacodes*); DNA from this species was recorded at all three sites, suggesting it could be a more common prey than suggested by hard-part analysis. Cartilaginous fish have not been documented in hard-part analysis and it was not known if this was due to their not being consumed or their cartilaginous skeletons not surviving digestion. We detected DNA in the seals faeces from the southern eagle ray (*Myliobatis australis*), a skate and two shark species, but only small numbers of sequences were recovered indicating these are minor prey items. In our DNA data, arrow squid stands out as the most prevalent invertebrate prey of Australian fur seals which is in agreement with hard-part analyses. Determination of the relative contribution of fish and squid using hard-part analysis is difficult since squid beaks are much more prevalent in regurgitates compared to faecal samples. We found arrow squid sequences constituted up to 15% of the mtDNA prey sequences (recorded at Seal Rocks). Further assessments are required to accurately monitor the relative contributions of squid to the seals diet, and may now be possible using DNA-based techniques. Finally, this study reveals geographic variations in Australian fur seal diet that have not previously been recognised, probably reflecting differences in prey availability. The general pyrosequencing approach presented significantly expands the capabilities of DNA-based methods of dietary analysis and is suitable for large scale diet investigations on a broad range of animals.

Details of this project are available in a paper to be published in Molecular Ecology in 2009 (accepted January 2009):

Deagle BE, Kirkwood R and Jarman SN (*In press*) Population level dietary analysis of Australian fur seals by pyrosequencing prey DNA in faeces

2. The Outcomes/Objectives

The degree to which the Activity has achieved the objectives
All Activities to be undertaken during this project have been completed. This includes the following milestones: • **Collection of samples** • **Extraction of DNA from tissues and mtDNA sequencing** • **DNA exclusion method developed** • **Meta-sample analysis and pyrosequencing** • **Development of software and analysis of data** • **Submission of scientific paper** **Two progress reports were also submitted and accepted by the Department (1st: 30 April, 2008 and 2nd: 21 July 2008).**

3. Appropriateness

The appropriateness of the approaches used in the development and implementation of the Activity
The approaches used in the implementation of the Activity were appropriate and represent a significant advance on previous method used in DNA-based analyses of diet.

4. Effectiveness

The degree to which the Activity has effectively met its stated objectives
The Activity has effectively met all stated objectives and managed to surpass several of the original objectives. For example, the initial proposal was for 200 samples from two seal colonies to be analysed, in fact 270 samples from three sites were processed. We also planned to obtain 9000 sequences; more than double this amount of data was obtained from the samples.