Australian Marine Mammal Centre

Final Report

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

- **Project No**. – 10/14
- **Title** - Combining genetics and morphology to resolve a longstanding taxonomic and conservation management issue: How many bottlenose dolphin species are there in Australian waters?
- **Chief Investigators** – Dr Catherine Kemper and Dr Michael Krützen
- **Organisation** – South Australian Museum

Activity Period – November 2010 to April 2012

Table of contents

1. Activity Summary
2. The Outcomes/Objectives
3. Appropriateness
4. Effectiveness
5. Financial Account of the Activity

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)
Successful wildlife conservation and management requires good baseline information on species identity and distribution. Bottlenose dolphins (*Tursiops* spp.) in Australian waters are subject to a suite of human impacts but because their population and species boundaries are unclear, managing these threats is difficult. By combining morphologic and genetic analyses of a large suite of bottlenose dolphin specimens from all around Australia, this study will investigates how many species there are and if there is evidence for population differences within species. Along with knowledge of the relative amount of threat for each species and population, the study will provide a sound basis for management by Commonwealth and State government. Osteological data (cranial and post-cranial measurements and characters) were collected to distinguish morphological groups using both traditional methods (2D linear cranial measurements) and a 3D digitiser (to analyse shape variation). About 250 bottlenose dolphin specimens, held in the major Australian museums, were measured. Examination of the type skulls of *T. truncatus* and *T. catalania* (both in British Museum of Natural History), *T. aduncus* (Museum für Naturkunde, Berlin), and *T. maugeanus* and *T. australis* (Queen Victorian Museum and Art Gallery, Launceston) was also undertaken to confirm the relationship of Australian material to type specimens.

The osteological data were analysed with multivariate statistics. Cluster analyses (hierarchical, k-mean) and discriminant function analyses of skulls produced two clusters for 2D methods, with a bit of cluster overlap. The measurements contributing most to the variation were the bigger length and width measurements. For 3D data, Principle Component Analysis showed that Australian bottlenose dolphin skull shape is different from other delphinid species (*Stenella* spp., *Delphinus delphis, Sousa chinensis, Lagenodelphis hosei* and *Steno bredanensis*), confirming *Tursiops* as a separate genus. The result also showed two adjacent clusters when looking at *Tursiops* spp. only, which were in concordance with the 2D analyses. Skull shape of putative *T. aduncus* had a rounder skull shape, a longer, narrower crania and rostrum than putative *T. truncatus*. Both 2D and 3D methods had affinities to the type specimens assigned to the *T. aduncus* (*T. aduncus* from the Red Sea, *T. catalania* from north Queensland) and *T. truncatus* (*T. truncatus* from England, *T. australis* and *T. maugeanus* from Tasmania) confirming the presence of at least two species in Australian waters. There was also preliminary evidence for sorting by geographic region, inshore and offshore forms.

For the genetic component of the project, about 660 tissue samples were successfully analysed for 19 microsatellite markers and the mitochondrial DNA (mtDNA) control region. The mtDNA data contained 146 unique haplotypes, of which about half were unique to a specific population or region and the rest shared between sampling regions around Australia. Neighbour-Joining, Maximum likelihood and Bayesian Inference clustering analyses suggests the existence of two distinct genetic clusters in Australian waters, corresponding to *T. aduncus* and *T. truncatus*. Structure analyses also showed evidence of these two distinct clusters. Further analyses will be conducted to measure genetic connectedness and gene flow between populations within species, and to assess the validity of the newly named bottlenose dolphin species, *T. australis*, from southeastern Australia.

2. The Outcomes/Objectives

List of the Project Objectives
1. Combine morphological and genetic data from Australian *Tursiops* in order to evaluate whether consistent and concordant differences are found between species/forms
2. Assess population genetic structure and gene flow between species/forms identified in 1.
The degree to which the Activity has achieved each of the objectives
Genetic analysis of soft tissue samples • All biopsy samples (n > 500) have been analysed at the CI Krützen lab in Zurich using 19 microsatellite markers and the mitochondrial DNA control region HVRI. • Soft tissue samples associated with museum specimens from SA (n > 100) have been analysed using the same marker set.

- Additional samples/DNA were obtained from Victoria (Charlton-Robe).

Collection of museum samples and morphological data from SA Museum

- Bone shavings and teeth were collected during 2011 by Jedensjo.
- Morphological data were collected during 2011 by Jedensjo.
- Progress report submitted.

Collection of museum samples and morphological data, other Australian museums

- Jedensjo travelled to all major Australian museums and obtained data from >250 skulls and skeletons.
- Morphological data were collected successfully from skulls held in Australian museums using traditional methods (2D) and the microscribe digitiser (3D). The specimens included *Tursiops* spp. and five other genera of delphinid cetacean (*Sousa*, *Stenella*, *Steno*, *Lagenodelphis* and *Delphinus*).
- Bone shavings and teeth were collected during 2011 by Jedensjo.

Analysis of osteological and external measurements

- 3D data has been analysed for *Tursiops* and other genera and the information presented at the Society for Marine Mammalogy conference in Tampa (Dec. 2011) and European Cetacean Society conference in (Galway)
- The analysis of 2D measurements has been achieved.
- It was decided that external measurements would not be studied because of time constraints and the difficulty in obtaining reliable and comparable data.
- MicroCT scans of a small sample of *Tursiops* earbones were carried out at the University of Adelaide microscopy centre. These will be used to test if morphological differences are present between *T. aduncus* and *T. truncatus*.

Genetic analysis of museum specimens collected by Jedensjo

- DNA was successfully extracted from bone and teeth genetic samples from the Museums ($n \approx 200$). Difficulties were encountered in obtaining results from the samples which led to delays of several months and in addition, more samples were obtained than anticipated.
- Bone and teeth samples are currently being analysed in the lab using the mitochondrial DNA control region HVRI and there are preliminary results available from the analysis of this data.

Measure and sample type specimens

- Jedensjo travelled to Berlin and London to measure and sample (bone shavings) the type specimens of *Tursiops truncatus* and *T. aduncus* and taxa that have been assigned to these species.
- We were very pleased to be given permission to obtain genetic samples from the type skulls of *T. truncatus* and this has not been done previously. The data obtained from these specimens will be crucial to interpreting the genetic results from Australian specimens.

Statistical analysis of genetic material

- Preliminary results were obtained during early 2012.
- Further analyses will be carried out during late 2012.

Prepare final report

- It is envisaged that marrying of morphological and genetic results will occur during late-2012 when Jedensjo is further advanced in the writing of her PhD.

3. Appropriateness

The appropriateness of the approaches used in the development and implementation of the Activity
The strength of this study has been the large sample size, distribution of samples around Australia and the breadth of techniques used. This was made possible due to the large collaboration we had with other researchers and museums in Australia. In this way, already collected biopsy samples could be reused and the data from the museums were easily collected covering very large parts of the Australian coast. Our goal was to incorporate as many people as we could, covering as much area as possible, and to use as many techniques as possible to optimise the outcome. We are confident that the outcomes are maximised by our approach.

4. Effectiveness

The degree to which the Activity has effectively met its stated objectives
Due to the large collaboration involved in this study, the sample collection of biopsy samples and museum specimens has been very efficient and effective. To clarify the taxonomic status of bottlenose dolphins in Australia, analysing all available data and using several techniques have been crucial and could not have been done differently. The project will meet its stated objectives but in a greater period of time than anticipated. The extremely large genetic samples size (n>700) has resulted in months of laboratory work making the suggested timetable unrealistic. All the genetic and morphological data have successfully been collected but the final statistical analysis for part of the project is still to be done during 2012/2013 in Jedensjo's final year of a PhD.

5. Communication

How results will be communicated to management
The results will be communicated through at least four scientific publications and presentations at several conferences. Jedensjo has presented papers at the Society for Marine Mammalogy conference in Tampa in December 2011 and the European Cetacean Society conference in Galway in March 2012. Jedensjo will submit a PhD thesis to the University of Zurich. The South Australian Museum will summarise the study on the research component of its website. It is hoped that some media interviews will be obtained when the study is finished.