

Australian Marine Mammal Centre Grants Program
Final Report
(subclause 9 and Schedule Item 5 of the Funding Agreement)

- **Project No.** 13/34
- **Title** - Consolidation and cataloguing of DPIPWE's cetacean biological sample collection
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- **Organisation** – Dept. Primary Industries, Parks, Water and Environment

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1. Project Summary

A clear, plain English summary of approximately 500 words outlining the work undertaken and any significant findings (for publication on the Department's web site) include what was done, why and the key findings resulting in recommendations summarised from the sections below.

This project sought to comprehensively curate and catalogue the >2000 cetacean biological samples collected and held by DPIPWE by systematically:

1. *verifying the status of samples;*
2. *improving storage of samples and sub-sample where appropriate;*
3. *updating associated meta-data and make summaries of the collection available via the ALA and AMMC databases.*

with the aim of facilitating access by the research community, helping to maximise the value of the collection, as well as improving efficiencies by potentially minimising unnecessary future sampling of live individuals or destructive sampling.

Setup: Our initial step was to service and upgrade existing facilities (dry storage, -20°C, -80°C and lab space) to support the verification and sub-sampling phases as well as to increase our long-term curation capacity. This involved creating additional physical storage space though concentrated efforts to organise and consolidate samples stored within the existing primary repository (a 39m³ -20°C freezer), the creation of additional archival shelving and storage systems and the procurement of sampling, sub-sampling and curation consumables.

Verifying, sub-sampling and curation: We obtained a comprehensive dataset of Tasmanian cetacean samples held by TMAG and AMMC and reconciled these with DPIPWE records. A full and systematic inventory of samples in DPIPWE storage was undertaken. As each sample was located, it was cross-checked against the DPIPWE database, data updated if

necessary, physical assessment of storage containers, replacement where necessary, replenish/replace storage media and relabelling performed. Sub-sampling of skin samples for TMAG voucher specimens and AMMC genetic samples was also undertaken at this time.

Data summaries and dissemination: We have produced a comprehensive verified data-set of Government cetacean biological sample holdings in Tasmania, including samples held by DPIPWE and TMAG. This data-set provides us with a valuable up-to-date record of sample holdings which will significantly increase the efficiency of sample use, and inform a more strategic and targeted approach to future sample collection.

The TMAG cetacean collection will be boosted by the provision of 695 voucher samples (and associated metadata), with these sub-samples forming the basis of an update of the Atlas of Living Australia by TMAG in May 2015. Duplicate genetic samples from 695 individuals will be presented to the AMMC to be stored as part of the Tasmanian cetacean DNA insurance archive.

The complete DPIPWE data-set will be provided to the National Marine Mammal Database to update existing records. DPIPWE's Natural Values Atlas (NVA) also has access to the data-set and it is on a list of scheduled NVA updates to be completed by the end of the current financial year. Both of these repositories provide publicly available meta-data summaries with more comprehensive details available from the data owner upon request

Summaries of sample holdings and of previous or ongoing research using Tasmanian cetacean samples will be made available through this report, and also included on the DPIPWE website. In addition, summaries of sample holdings will be directly disseminated to management agencies and research institutions to raise awareness of the collection and improve its accessibility for research activities.

KEY FINDINGS:

1. In total, the verified Tasmanian Government cetacean sample holdings consist of **3692 biological samples**, sourced from **29 species** and **1512 individuals** (Appendix 1 and 2);
2. **695 sub-samples** from individual cetaceans will be provided each to TMAG (voucher) and AMMC (genetic sample insurance);
3. The DPIPWE working data-set will be backed up by adding meta-data summaries to publicly available State and National databases (i.e. the Tasmanian NVA and National Marine Mammal Database);
4. New historical information has been added to the Tasmanian stranding record (23 stranding records from pre-1900, and 18 records from 1901-1970);
5. Data will be disseminated directly to relevant management authorities and research institutions.

RECOMMENDATIONS:

1. Dissemination of data-sets and summaries should be ongoing where opportunities arise, for example: dissemination via an International Whaling Commission paper and the Government Cetacean Management Workshop;
2. Data-set back-ups should be renewed regularly through provision of updated data-sets to the NMMD and NVA;

3. Sample holdings should be regularly backed-up through provision of sub-sampled voucher specimens and genetic insurance samples to the TMAG and AMMC when new samples are collected;
4. Current sample holdings and collection activities should be reviewed to identify key gaps in sample availability and to inform a more strategic and targeted approach to future sample collection.
5. As well as dissemination of the data-set, opportunities for research using samples held in the Tasmanian Government collection should be identified, and potential collaborators directly approached.

2. The Outcomes and Objectives – Key Findings

List the Project Objectives and address each one, noting the degree to which the objective was achieved through the research and issues that may have hampered its success. Describe the key findings as they relate to the objectives and the management questions identified in the initial application.

The key Project Objectives were:

1. Ensure the Tasmanian Government cetacean biological sample collection is secure into the long-term;
2. Assess and improve the accuracy and resolution of the associated data;
3. Improve the accessibility of the sample collection for research activities.

1. Ensure the sample collection is secure into the long-term.

The work undertaken through this project has resulted in effective long-term storage and curation solutions for the Tasmanian cetacean biological sample collection. The increased capacity built into the collection facilities, validation and accurate curation of samples, replenishment of storage medium for these samples, and distribution of duplicate samples across several collection archives has resulted in improved preservation and sample security for the future.

The Tasmanian Government sample holdings will continue to be available for research that will contribute to a range of national and international policies and conservation initiatives, including but not restricted to:

- Provide information on population structure and connectivity, as well as spatial and temporal aspects of species occurrence;
- Contribute information directly to the development and/or review of Conservation Management Plans for threatened cetacean species; and,
- Improve the response to and management of cetacean strandings, and contribute to the understanding of these events. Information gained will inform the National Guidance on the Management of Whale and Dolphin Incidents in Australian Waters, along with state agency policies and protocols for stranding management.
- Add to the wealth of non-lethal research on wild cetaceans, a core element of Australia's reporting and representation to the IWC.

2. Improve the accuracy and resolution of the associated data.

The Tasmanian Government cetacean sample collection holds nearly 3700 individual samples, with the majority of tissue samples collected during the last 30 years. Fluctuating

resourcing levels has resulted in varying levels of curation. By reconciling each individual sample against the DPIPWE database records we have addressed discrepancies and data gaps, thereby verifying the complete sample collection against the database to ensure accurate and up to date curation.

We have reconciled both the TMAG and AMMC databases (and associated sample collections) against our own to ensure we hold accurate and current meta-data on the provenance and storage of these samples, distribution of duplicate sub-samples for voucher and insurance purposes, and the research collaborations involved. Previously unconsolidated historical information contained in TMAG records has been added to the official Tasmanian stranding record, including 23 records from pre-1900, and 18 records from 1901-1970.

The DPIPWE and TMAG sample collections typically serve different purposes and are the result of distinct functions for each agency. TMAG typically holds limited samples from each individual and samples are held mainly for reference and voucher purposes. DPIPWE however typically collects and stores samples for investigation and research objectives. This is reflected in the breakdown of samples held by each agency: DPIPWE holds 3079 samples from 892 individuals (average of over four samples from each animal) across 25 species, whereas TMAG holds 1270 samples from a total of 1133 individuals (average 1.1 sample per animal) across 28 species (see Appendix 1 and 2 for a summary of verified DPIPWE and TMAG sample holdings).

3. Improve the accessibility of the sample collection for research activities.

Improving the accessibility and awareness of the Tasmanian Government's cetacean biological sample holdings to the broader research community is critical to maximising the value of samples and validating sample collection activities. An important aspect of this is also generating awareness of sample analyses that have already been undertaken. This supports Australia's Research Priorities for Cetaceans that encourage the use and development of non-lethal research techniques for studying whales.

We will increase the access that researchers and other potential collaborators have to our sample collection through the web publication of up-to-date comprehensive meta-data summaries of cetacean biological samples held by the Tasmanian Government. Meta-data summaries will be added to the National Marine Mammal Database by DATE. DPIPWE's Natural Values Atlas (NVA) has access to the data-set and has included this as one of several updates to the NVA scheduled to be completed by the end of the current financial year. Summaries of sample holdings are made available through this report (see Appendices), and will also be included on the DPIPWE website.

A summary of previous or ongoing research using samples obtained through the Tasmanian Government sample holdings is also provided in this report (see Appendix 3). It is anticipated that this will assist in minimising unnecessary and inefficient duplication of effort or informing important supplement studies.

In addition, accurate summaries of current sample holdings will be directly disseminated to management agencies and research institutions to raise awareness and improve the accessibility of the sample collection for research activities.

The project has also facilitated improvements to sampling and curation equipment and procedures at the primary DPIPWE storage facility at Taroona. This has increased the capacity for long-term preservation of samples, and will greatly enhance the efficiency of locating and extracting samples when required.

3. Implications for Management

What are the key recommendations for management based on the findings.

Strategic sampling of biological material from stranded marine mammals has the potential to contribute extensively to our understanding of these elusive individuals and populations. A key recommendation from our work is that the effective dissemination of such collections is a critical component to maximise the value of such collections as well as improving efficiencies by potentially minimising unnecessary future sampling of live individuals or destructive sampling.

Through this project we have identified and sub-sampled tissue from 695 individual cetaceans and provided these tissue samples to TMAG and AMMC. Timely analysis of these samples will provide significant data to directly address one of the AMMC's current research priorities: *Quantify population status, structure, distribution, habitat use and ecological influences for marine mammal populations for which such data can demonstrably inform and improve conservation management.*

Consolidation of data held by DPIPWE and TMAG during the project has resulted in an additional 41 stranding events added to the official Tasmanian stranding record, improving the accuracy of ongoing analysis of spatial and temporal patterns in stranding occurrence aimed at enhancing our understanding of these events and DPIPWE's preparedness for response.

When disseminated, the meta-data and collection summary outputs resulting from the validation and verification of our sample catalogue will have considerable potential to contribute to a range of national and international management policies and conservation initiatives.

4. Other Benefits

How has this project advanced the field of research? (e.g. scientific discoveries, new methodologies)

Through the consolidation and verification of our existing samples and catalogues we are able to direct and refine our sampling protocols and practices. This ensures that we are continuing to build on our existing sample types and research focus, while maximising the opportunities to contribute to future research prioritise.

We anticipate that as the metadata from our collection becomes more widely circulated and available to other research groups, that we will be able to continually refine and target our sampling to accommodate and facilitate further research where there are key knowledge gaps. Particular focus will be research that improves our understanding of cetacean biology and ecology, and so inform improved management and conservation.

Enhanced awareness of the sample collection, as well as previous/ongoing research using samples derived from this collection, will minimise unnecessary and inefficient duplication of efforts toward sampling and analyses.

5. Problems Encountered (if any)

Describe any major problems encountered during the Activity and how they were

addressed.

Due to organisational restructures within DPIPWE, we faced delays in project commencement attributed to staff movements and re-deployments. Although these problems contributed to delays upon our progress milestones and our anticipated rate of expenditure. We addressed delays by engaging professional and contract services to assist in key targeted areas and have been granted an extension on funding expenditure.

6. Communication

How will results be communicated to management

The complete meta-data set will be publically available through a number of avenues. Meta-data summaries will be added to the NMMD and the NVA. Summaries of sample holdings and of previous or ongoing research using Tasmanian cetacean samples will be made available through this report, and also included on the DPIPWE website.

In addition, summaries of sample holdings will be directly disseminated to management agencies and research institutions will be ongoing where opportunities arise to raise awareness of sample availability, and to improve and help facilitate access to the sample collection for research activities that inform management, for example: dissemination via an International Whaling Commission paper and at the annual Government Cetacean Management Workshop.

Opportunities for management-based research using samples held in the Tasmanian Government collection will be identified, and potential collaborators directly approached.

Stakeholder engagement feedback (plain English for feedback to stakeholders)

Students supported (if any)

Several student projects are currently underway that utilise cetacean biological samples and metadata associated with this project – See appendix 3.

PhD Theses and dissertations (if any)

Several PhD projects are currently underway that utilise cetacean biological samples and metadata associated with this project – See appendix 3.

Publications (other than theses and dissertations)

Planned publications

A short note to IWC summarising the project and the resulting sample collection and meta-data

Presentations


7. Project Outputs

A list of the actual outputs of the research including milestones, progress reports and data products such as models etc.	Proposed date of completion	Actual date of completion
AMMC project 13/34 progress report	Oct 2014	Oct 2014

Curation and validation of existing sample collection	Jul 2014	Feb 2015
Update DPIPWE marine mammal database	Feb 2015	Mar 2015
Voucher Specimens to TMAG	Sep 2014	April 2015
Insurance genetic samples to AMMC	Oct 2014	TBA
Summary of previous and current use of biological sample data	Nov 2014	Jan 2015
Final report to AMMC	April 2015	TBA
Publicly available sample collection metadata	April 2015	TBA

8. Financial Account of the Activity

Include reasons for any variation to the budget, underspends and difficulties
An interim financial account of the activity is attached.

Signature of Chief Investigator	
Name	Rachael Alderman
Date	30/04/2015
Signature of Organisation Representative	
Name	
Date	

Please forward 1 hard copy, and one electronic Word document of this report to:

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Appendix 1: Verified cetacean samples held in the DPIPWE collection (from 892 individuals) – table continues over page.

Species	Common name	Sample Type															
		Baleen	Blubber	Brain	Kidney	Liver	Lung	Heart	Muscle	Skin	Skull	Stomach	Testis	Dorsal fin/flukes	Ear bone		
<i>Balaenoptera acutorostrata</i>	Minke whale		3														
<i>Balaenoptera bonaerensis</i>	Antarctic Minke Whale		4														
<i>Balaenoptera edeni</i>	Bryde's whale	1	1														
<i>Caperea marginata</i>	Pygmy right whale	2	20		4	4					4	1	1				
<i>Delphinus delphis</i>	Common dolphin		63		17	20					16	4					6
<i>Eubalaena australis</i>	Southern right whale	2	3		1	1											
<i>Globicephala melas</i>	Long-finned pilot whale		681		7	7					84						
<i>Grampus griseus</i>	Risso's dolphin		23	2	8	3					8						
<i>Hyperoodon planifrons</i>	Southern bottlenose whale		0														
<i>Kogia breviceps</i>	Pygmy sperm whale		7		2	2					2	1	1				
<i>Lagenorhynchus obscurus</i>	Dusky dolphin		4		1	1											
<i>Lissodelphis peronii</i>	Southern right whale dolphin		3														
<i>Megaptera novaengliae</i>	Humpback whale		9		2	2											
<i>Mesoplodon bowdoini</i>	Andrew's beaked whale		6		3	3						2	2	1			
<i>Mesoplodon densirostris</i>	Blainville's beaked whale		0														
<i>Mesoplodon grayi</i>	Gray's beaked whale		9			1					3	1	3				
<i>Mesoplodon hectori</i>	Hector's beaked whale		0														
<i>Mesoplodon layardii</i>	Strap-toothed beaked whale		19		5	6					2			1	2		
<i>Physeter macrocephalus</i>	Sperm whale		160		1	5									7		
<i>Stenella coeruleoalba</i>	Striped dolphin		0														
<i>Tasmacetus shepherdi</i>	Shepherd's beaked whale		3		1	1						1					
<i>Tursiops truncatus</i>	Bottlenose dolphin		44		5	5					2				2		
Unidentified Delphinidae	Unidentified dolphin		1			1											
Unidentified Ziphiidae	Unidentified beaked whale		1		1	1											
<i>Ziphius cavirostris</i>	Cuvier's beaked whale		10		3	3											
Grand total		5	1074	2	61	71	9	5	449	863	2	128	9	9	6		

Appendix 3: Summary of previous and ongoing research and analyses utilising samples sourced from the Tasmanian Government cetacean sample collection, separated by individual study.

SPECIES	ANALYSIS	PURPOSE	SAMPLE TYPE USED	N INDIVIDUALS	N SAMPLING EVENTS	DPIPWE D'BASE STRANDING ID	CETACEAN ID (BIOPSIES)	FURTHER INFORMATION
Andrews Beaked whale (Mesoplodon bowdoini)	Genetic sequencing (mtDNA control region)	Species and sex confirmation	Skin	1	1 stranding	1137373458		Internal DPIPWE report
Antarctic minke whale (Balaenoptera bonaerensis)	Genetic sequencing (mtDNA control region)	Species confirmation	Skin	2	2 strandings	1383272988, -1209325878		Internal DPIPWE report
Blue whale (Balaenoptera musculus)	Genetic sequencing (mtDNA control region)	Species and sex confirmation	Skin	2	2 biopsies		172303026 and 1404888341	Internal DPIPWE report
Bottlenose dolphin (Tursiops truncatus)	Genetic sequencing (mtDNA control region)	Species identification	Skin	42	1 stranding	1276838809		Internal DPIPWE report
	Genetic sequencing (mtDNA control region)	Phylogenetic analyses	Skin	13	13 biopsies		TTRU_220508_FRE and TADU_260808_FRE_01	Results Pending
	Heavy metal analysis	Determine heavy metal concentration in stranded dolphins	Liver and kidney	24	1 stranding	1276838809		Internal DPIPWE report
	Genetic analyses	Phylogenetic analyses	Skin	4	3 strandings	1294354660 (x2), 1300156674, -1197645619		Results Pending
	Morphology	Phylogenetic analyses	Skull	1	1 stranding	-1717265975, -1717265975		Results Pending
	Diet analysis	Investigate diet of T. truncatus stranded in Tasmania	Stomach contents	3	3 strandings	124, 158		Gales et al. 1992
Bryde's whale (Balaenoptera edeni)	Genetic sequencing (mtDNA control region)	Species and sex confirmation	Skin	1	1 stranding	1360814277		Internal DPIPWE report

SPECIES	ANALYSIS	PURPOSE	SAMPLE TYPE USED	N INDIVIDUALS	N SAMPLING EVENTS	DPIPWE D/BASE STRANDING ID	CETACEAN ID (BIOPSIES)	FURTHER INFORMATION
Common dolphin (<i>Delphinus delphis</i>)	Genetic sequencing (mtDNA control region and 14 microsatellite loci)	Population structure and management units of D. delphis subject to fisheries bycatch in southern Australia	Skin	15				Biglmann et al. 2008, GenBank
	Genetic sequencing (mtDNA control region and 7 microsatellite loci)	Population structure of D. delphis in southern Australia	Skin	20	8 strandings			Biglmann et al. 2014, GenBank
Dwarf minke whale (<i>Balaenoptera acutorostrata</i>)	Visual	Species identification and museum collection	Baleen	1	1 stranding	58		sample lodged SA museum
Gray's beaked whale (<i>Mesoplodon grayi</i>)	Genetic sequencing (mtDNA control region)	Species confirmation	Skin	4	4 strandings	1130108616, -1078399311, -1869076667, 1406257544		Internal DPIPWE report
	Genetic sequencing	Gray's beaked whale genetics	Skin	2	2 strandings	1330907007, 463976401		GenBank
	Genetic sequencing (mtDNA control region)	Species and sex confirmation	Skin	1	1 stranding	-413058960		Internal DPIPWE report
Hector's beaked whale (<i>Mesoplodon hectori</i>)	Genetic sequencing (mtDNA control region)	Species and sex confirmation	Skin	1	1 stranding	64358151		
Humpback whale (<i>Megaptera novaeangliae</i>)	Genotyping (10 microsatellite loci)	Genetic differentiation of Australian humpback whale populations	Skin	70	70 biopsies			Schmitt et al. 2014

Stable isotopes	Insight into feeding location, fasting duration, duration of maternal provisioning and weaning	Baleen	2	2 strandings	555544922, 914545423	Results Pending
Long-finned pilot whale (<i>Giubicephala melas</i>)	Organic pollutant testing	Blubber	1	1 stranding	94	Symons et al. 2003
	Genetic sequencing (mtDNA control region)	Skin	215	6 strandings	94, 1046605252, 1072054000, 1103089108, 1179807296	Oremus et al. 2009, GenBank
	Molecular determination of gender	Skin	5	1 stranding	1231450668	Gales et al. 2012
	Genetic sequencing (mtDNA control region)	Skin	215	5 strandings	94, 1046605252, 1072054000, 1103089108, 1179807296	Oremus et al. 2013
	Organic pollutant testing	Blubber	115	4 strandings	1231450668, 1237951211, 1296184696, 1300746185	Weijs et al. 2013
	PCB bioaccumulation and pharmacokinetic modelling	Blubber	38	2 strandings	1231450668, 1237951211	Weijs et al. 2014
	Investigation of dentine layers in stained and sectioned teeth	Teeth	21	1 stranding	1836352224	Results Pending
	Diet analysis	Stomach contents	2	2 strandings		Gales et al. 1992
Pygmy right whale (<i>Caperea marginata</i>)	Genetic sequencing (mtDNA control region)	Skin	2	1 stranding	-1361100797	Internal DPI/PWE report
	Visual	Skeleton	1	1 stranding	447	Lodged in SA Museum
	Iron concentration	Faecal matter	1	1 stranding	453	Results Pending

Pygmy sperm whale (<i>Kogia breviceps</i>)	Diet analysis	Investigate diet of K. breviceps stranded in Tasmania	Stomach contents	2	1 stranding	91	Beasley et al. 2013
Risso's dolphin (<i>Grampus griseus</i>)	Diet analysis	Identification of stomach contents as part of stranding investigation	Stomach contents	3	3 strandings	1416801980, - 1089844449, - 1083504254	Results Pending
	Biotoxin and heavy metal testing	Investigation of cause of large-scale mortality event	Liver and kidney	9	9 strandings	1416801980, - 1773397124, - 1083504254, 1422326684, 1422323656, - 308267575, - 785566603, - 650004344, 1089844449,	Results Pending
Southern bottlenose whale (<i>Hyperoodon planifrons</i>)	Genetic sequencing (mtDNA control region)	Species and sex confirmation	Skin	1	1 stranding	1398664728	Internal DPIPWE report
Southern right whale (<i>Eubalaena australis</i>)	Genetic sequencing (mtDNA control region and 13 microsatellite loci)	Population structure of southern right whales in Australia and NZ	Skin	3	3 biopsies	TAS0701, TAS0702, TAS0703	Internal DPIPWE Report
	Genetic sequencing (mtDNA control region and 13 microsatellite loci)	Global population structure and connectivity of southern right whales and fidelity to feeding grounds	Skin	7	7 biopsies	TAS1207, TAS1201, TAS1210, TAS1215, TAS1213, TAS1231, TAS1232	Results Pending
	Stable isotopes	Fidelity to feeding grounds	Skin	7	7 biopsies	TAS1207, TAS1201, TAS1210, TAS1215, TAS1213, TAS1231, TAS1233	Results Pending
	Iron concentration	Iron storage and defecation in cetaceans	Faecal matter	1	1 stranding	1406249150	Results Pending
	Genetic sequencing (mtDNA control region)	Species and sex confirmation	Skin	7	7 biopsies	TAS1207; TAS1201 TAS1210; TAS1215; TAS1213; TAS1231; TAS1232	Internal DPIPWE report

Southern right whale dolphin (<i>Lissodelphis peroni</i>)	Next generation sequencing	Cetacean phylogeny	Skin	1	1 strandings	1094616663	Results Pending
Sperm whale (<i>Physeter macrocephalus</i>)	Organic pollutant testing	Identification of POPs in stranded Tasmanian cetaceans	Blubber	7	2 strandings	86, -770927380	Symons et al. 2003
	Genetic sequencing (mtDNA control region and 13 microsatellite loci)	Population structure of sperm whales	Skin	48	2 strandings	ID -2070887088, -72150957	Results Pending
	Epigenetic aging	Molecular age estimation	Skin	12	2 strandings	ID -2070887088, -529782718	Results Pending
	Investigation of dentine layers in stained and sectioned teeth	Estimation of age, and variation in energetic and trophic variability during lifetime	Teeth	37	9 strandings	86, -770927380, 2132258891, 1387505334, 1436809230, -529782718, 1154648229, 2139071659, -1928265777, 127	Results Pending
Strap-toothed beaked whale (<i>Mesoplodon layardii</i>)	Genetic sequencing (mtDNA control region)	Species and sex confirmation	Skin	1	1 strandings		Internal DPIPWE report
	Genetic sequencing (mtDNA control region)	Species and sex confirmation	Skin	2	2 strandings	725423404, -1317880215	Internal DPIPWE report