

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

- **Project No.** – Project #8 (2009)
- **Title** - Population size of blue whales in Australian waters
- **Chief Investigator** – Dr Luciana Möller
- **Organisation** – Flinders University

Activity Period – October 2010 – April 2011

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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)

Boat surveys for photo-identification and biopsy sampling of blue whales were undertaken in the Bonney Upwelling (VIC and SA), Perth Canyon (PC) and Geographe Bay (WA) in 2009 and 2010. During these surveys 69 individual blue whales were photo-identified and 40 were biopsied in these areas. Photo-identification analysis and matching within and between seasons, and within and between areas, suggest short to medium term residency of blue

whales within an area within a given season (few days to about a month). It also suggests that blue whales may move between the areas within a given season and between seasons. They were also found to revisit the same areas between seasons. We have previously shown that the two Australian feeding aggregations of pygmy blue whales in the Bonney Upwelling and Perth Canyon constitute the same genetic population (Attard et al. 2010), however the subspecific and population identity of blue whales that travel through Geographe Bay was unknown. Genetic analysis as part of this project suggests that Geographe Bay blue whales are also from the pygmy blue whale subspecies, and likely belong to the same genetic population as the two Australian feeding aggregations. In addition, comparisons with Antarctic blue whale samples suggest that whales from the Australian feeding aggregations and Geographe Bay blue whales are part of one genetic cluster, which is separate from Antarctica blue whales. However, there was also genetic evidence in our dataset that the two subspecies hybridise and migrants can be found outside the expected geographic location of the respective subspecies. Our results suggest that the panel of genetic markers that were developed as part of this project is a powerful tool for determining subspecies identity of blue whale populations and detecting migrants and admixed individuals between the two Southern Hemisphere subspecies.

2. The Outcomes/Objectives

List of the Project Objectives

The overall aim of the project was to investigate the **abundance** of blue whales in Australian aggregation areas using mark-recapture techniques based on photo- and genetic-identification.

The objectives of biopsy sampling and **genetic-identification** were to (1) allow genetic mark-recapture of individuals and (2) determine subspecies and population origin of individuals to refine population estimates (including population genetic identity of Geographe Bay blue whales).

The objectives of **photo-identification** were to (1) allow photo-ID mark-recapture of individuals and (2) compile life histories to examine site fidelity, associations and document movements of blue whales between Australian areas.

The degree to which the Activity has achieved each of the objectives

Genetic identification

Biopsy sampling and genetic markers

In the 2010 season, 16 individuals from the Perth Canyon, 11 individuals from the Bonney Upwelling, and 6 individuals from Geographe Bay were biopsy sampled.

Genetic markers utilised were 21 microsatellite loci and mtDNA control region sequences. Individuals were removed from microsatellite analyses if there were greater than 3 microsatellite loci with missing data. The microsatellites showed no evidence for null alleles, stutter bands or short allele dominance, and no significant deviations from Hardy-Weinberg equilibrium or evidence of linkage disequilibrium, based on Australian feeding aggregation samples (n = 101) known to constitute one genetic population (Attard et al. 2010).

Genetic mark-recapture

Microsatellite analyses showed that within the 2010 season, one individual was re-sampled 5 times at the Bonney Upwelling. There were no resamples between the 2010 samples (n = 27) and pre-2010 Australian samples (n = 74) or Antarctic samples (n = 99). Two between-year genetic recaptures (that do not involve 2010 samples) within the Perth Canyon, which were not found during initial photo-ID analysis, were confirmed by photo-ID based on the prior knowledge that these were genetic recaptures. This emphasises the benefit of using both photo-ID and genetic methods in mark-recapture studies of blue whales.

Geographe Bay genetic identity

We have previously shown that the two known Australian feeding aggregations of blue whales constitute the same genetic population (Attard et al. 2010), however the subspecific and population identity of blue whales that travel through Geographe Bay is unknown. The number of individuals biopsied from Geographe Bay in 2009 (funding from AMMC project 0809/13) and 2010 (this project) is 13 (due to the lower than expected number of sightings in both years).

Fixation indices based on microsatellite loci calculated in ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010) showed no significant evidence of genetic differentiation between Geographe Bay and the Australian feeding aggregations ($F_{ST} = -0.009$, $P = 0.983$), and significant evidence of genetic differentiation between Geographe Bay and Antarctica ($F_{ST} = 0.108$, $P < 0.001$). A power analysis in POWSIM (Ryman and Palm 2006) indicated that a microsatellite $F_{ST} \geq 0.016$ between the Australian feeding aggregations and Geographe Bay could be detected with $\geq 95\%$ confidence (96.3% Fisher's exact test, 99.1% chi-square). The Australian feeding aggregations also had a similar, significant level of genetic differentiation to Antarctica ($F_{ST} = 0.122$, $P < 0.001$). MtDNA control region sequencing is currently in progress for Antarctica, however all sequenced

Geographe Bay individuals (n = 7, remainder currently being sequenced) share haplotypes with the Australian feeding aggregations. Similarly, STRUCTURE 2.3.3 (Hubisz et al. 2009) (admixture model, with and without using sampling locations as priors, correlated allele frequency model) detected 2 genetic clusters (using the delta-K (Evanno et al. 2005) method). The Australian feeding aggregations and Geographe Bay blue whales were grouped as one genetic cluster, and Antarctica as a separate genetic cluster.

This together indicates that Geographe Bay blue whales are from the pygmy blue whale subspecies, and likely belong to the same genetic population as the Australian feeding aggregation blue whales (with enough power to detect an $F_{ST} \geq 0.016$). It also shows that relatively few samples are required to determine subspecies identity of blue whale populations when utilising this powerful, 21 microsatellite marker set.

Subspecies identity

STRUCTURE and GENECLASS 2.0 (Piry et al. 2004) were utilised to detect migrants or admixed individuals from the two Southern Hemisphere subspecies. Based on genetic structure results (see above), the Australian feeding aggregations and Geographe Bay were grouped together as one Australian genetic population for these tests.

The GENECLASS exclusion test determines whether an individual can be excluded from belonging to a population (their sampled population or otherwise). Since each population is tested separately, this does not assume that all populations have been sampled. All except two individuals sampled in Antarctica were excluded from the Australian blue whale population and three Australian samples (all from Perth Canyon) were excluded from the Antarctic population ($P < 0.05$).

GENECLASS and STRUCTURE both detected migrants, and STRUCTURE also detected 1st generation admixed individuals (GENECLASS is not capable of detecting admixed individuals) (Table 1, Figure 1). The results of both programs agree when the different assumptions of the analyses are considered. For instance, GENECLASS L_h criterion does not assume that all populations have been sampled, unlike STRUCTURE and the GENECLASS L_i/L_{max} criterion. This means L_h will not classify migrants from unsampled populations as residents, unlike L_i/L_{max} criterion and STRUCTURE, however the L_h criterion has less power than the L_i/L_{max} criterion.

The results reveal the 21 microsatellite markers as a powerful tool to determine subspecies identity, and will be very important in future mark-recapture studies given that the evidence suggests that the subspecies hybridise and migrants can be found outside the expected geographic

location of their subspecies. Additionally, the evidence suggests that a currently undescribed genetic population may also migrate to Australia and Antarctica.

Table 1: Individuals detected as migrants using GENECLASS, and migrants or admixed individuals using STRUCTURE. A tick indicates significant evidence of migrants or admixture, except for the exclusion test where it indicates that the sample was excluded from their sampled population.

Sampling location	ID	STRUCTURE		GENECLASS		
		$\nu = 0.05$	$\nu = 0.01$	Exclusion test	L_{ij} criterion	L_{ij}/L_{max} criterion
Australia	1			✓*	✓*	
	2				✓*	
	3				✓*	
	4			✓* (& excluded from Antarctica)	✓**	
	5				✓*	
	6			✓*	✓*	
Antarctica	7	✓** (hybrid)				
	8				✓*	
	9	✓*** (migrant)	✓*** (migrant)			✓***
	10	✓*** (hybrid)	✓*** (hybrid)			
	11	✓*** (migrant)	✓*** (migrant)			✓***
	12	✓*** (hybrid)	✓** (hybrid)			
	13	✓*** (hybrid)	✓*** (hybrid)			

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

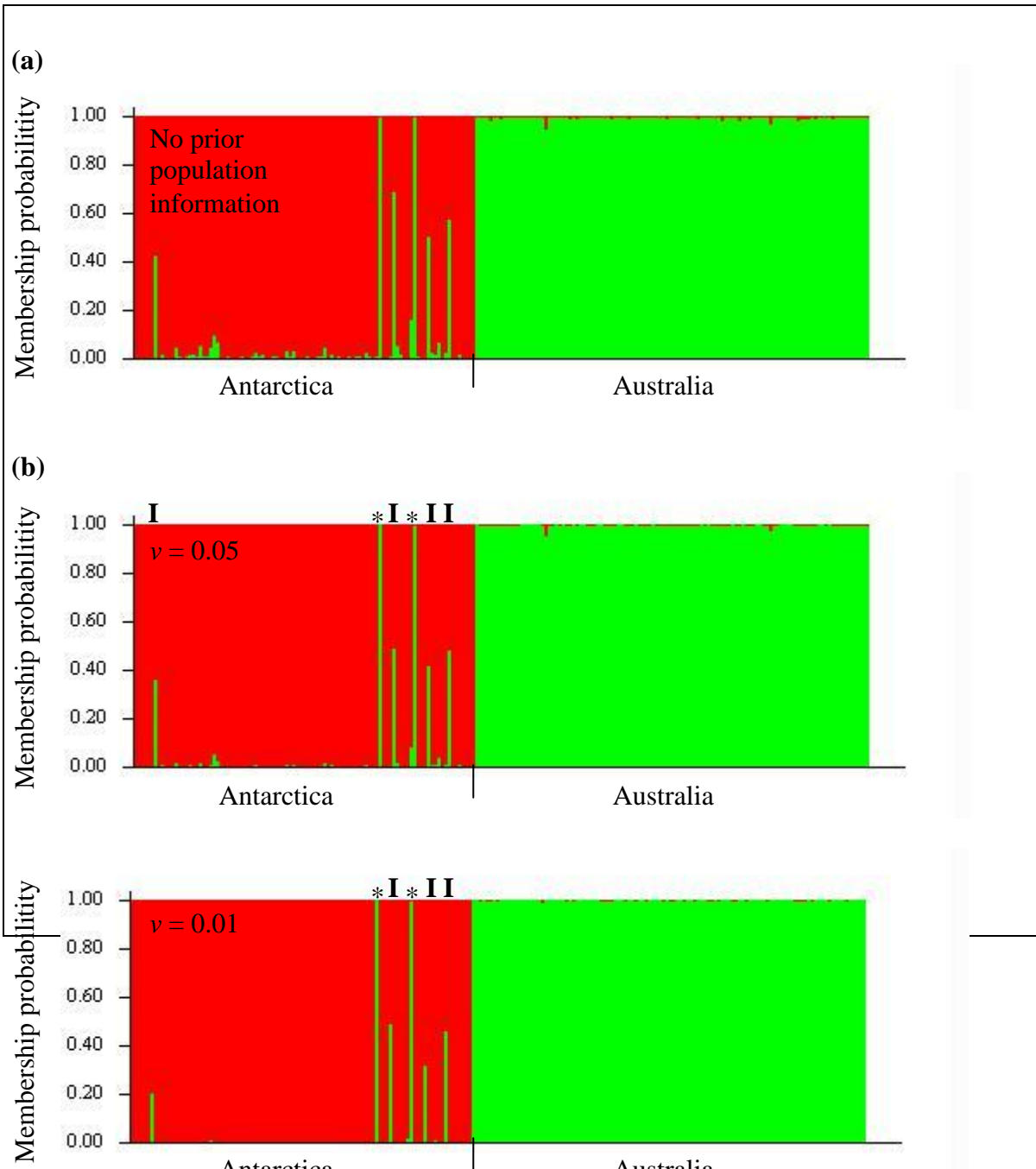


Figure 1: Clustering results of STRUCTURE analysis (a) without prior population information and (b) using prior population information to test for migrants and admixed individuals (* = migrant, **I** = 1st generation hybrid).

Future work

We have successfully answered this project's objectives, however we are currently conducting further data collection and analyses that will add further information to these objectives. The mtDNA control region sequencing for Antarctic samples is still under progress, and 10 Antarctic samples remain to be genotyped at microsatellite loci. This was delayed as the final consignment of IWC Antarctic samples required to determine subspecific identity were received in February 2011 (~1.5 years after access was originally granted at the 2009 IWC Sub Committee meeting) due to circumstances out of our control. Additionally, a simulation approach (e.g. Schwartz and Beheregaray 2008) will be conducted to evaluate the statistical power of identifying migrants or admixed individuals between subspecies using a combination of HYBRIDLAB 1.0 (Nielsen et al. 2006), NEWHYBRIDS 1.0 (Anderson and Thompson 2002) and STRUCTURE.

Photo-identification

Total number of whales identified in 2009 and 2010 (Geographe Bay, GB), and 2010 (Bonney Upwelling, BU and PC): the total number of whales identified was 69, including 23 individuals in GB, 23 individuals in BU and 23 individuals in PC.

Photo-identification analysis was performed as show in Table 1a,b below. Centre for Whale

Research (CWR) represents photographs from PC, Western Whale Research (WWR) represents photographs obtained in GB. GB photographs taken in 2010 and BU photographs taken prior to 2010 are still under analysis.

Table 1a. Photo-identification analysis performed within and between blue whale catalogues (prepared by V. Boladeras, CWR).

	CWR 2010		WWR 2009		BU 2010	
	Checked	# of people checked	Checked	# of people checked	Checked	# of people checked
CWR Entire Catalogue	<input checked="" type="checkbox"/>	3	<input checked="" type="checkbox"/>	3	<input checked="" type="checkbox"/>	3
WWR Entire Catalogue	<input checked="" type="checkbox"/>	3	<input checked="" type="checkbox"/>	3	<input checked="" type="checkbox"/>	3
BU 2010	<input checked="" type="checkbox"/>	3	<input checked="" type="checkbox"/>	3	<input checked="" type="checkbox"/>	3

	CWR 2005 - 2009		WWR 2005 - 2008 (new entries)		WWR 1994 - 2003	
	Checked	# of people checked	Checked	# of people checked	Checked	# of people checked
CWR Entire Catalogue	<input checked="" type="checkbox"/>	2	<input checked="" type="checkbox"/>	3*	<input checked="" type="checkbox"/>	2*
WWR Entire Catalogue	<input checked="" type="checkbox"/>	2	<input checked="" type="checkbox"/>	3	<input checked="" type="checkbox"/>	2
BU 2010	<input checked="" type="checkbox"/>	3	<input checked="" type="checkbox"/>	3	<input checked="" type="checkbox"/>	3

* = partial

Table 1b. Photo-identification analysis performed within the blue whale BU catalogue (prepared by P. Gill, Blue Whale Study).

Whale ID	Sighting ID	Date	Area	Days between resights
Not assigned yet	116	20-Mar-09	Bonney Upwelling	
Not assigned yet	126	27-Mar-09	Bonney Upwelling	7
Not assigned yet	117	20-Mar-09	Bonney Upwelling	
Not assigned yet	132	12-Apr-09	Bonney Upwelling	23
Not assigned yet	125	27-Mar-09	Bonney Upwelling	
Not assigned yet	91	24-Mar-10	Bonney Upwelling	362
Not assigned yet	21	27-Jan-06	Bonney Upwelling	
Not assigned yet	24	3-Feb-06	Bonney Upwelling	7
Not assigned yet	35	5-Feb-06	Bonney Upwelling	2
Not assigned yet	130	31-Mar-09	Bonney Upwelling	1149
Not assigned yet	75	13-Mar-10	Bonney Upwelling	
Not assigned yet	82	14-Mar-10	Bonney Upwelling	1
Not assigned yet	88	15-Mar-10	Bonney Upwelling	1

Re-sightings within the areas during the 2009/2010 season: There was no re-sighting of whales in GB. In PC one whale was re-sighted after four days of its initial identification. In BU four whales were re-sighted, with the number of days between sightings ranging from one to 23 days (table 1b).

Re-sightings between the areas during the 2009/2010 season (excluding GB 2010): One whale photo-identified in GB in 19/12/09 was re-sighted in BU in 13/03/10.

Re-sightings between seasons (please note that the AMMC did not fund this component): Summary of re-sightings is presented in the Table 2 below and Table 1b above (for BU 2009/2010 photos). Note that for between area comparisons only 2010 BU photos were included for analysis,

and for GB only photos up to 2009.

Table 2. Summary of blue whale re-sightings from the 2009/2010 season matched within and between photo-identification catalogues. Whale ID obtained from CWR catalogue (prepared by V. Boladeras, CWR).

<u>Whale ID</u>	<u>Sighting ID</u>	<u>Date</u>	<u>Area</u>	<u>Days between resights</u>
WA0123	156	6/03/2004	Perth Canyon	-
WA0123	159	9/03/2004	Perth Canyon	4
WA0123	332	16/04/2010	Perth Canyon	2233
WA0123	341	18/04/2010	Perth Canyon	2235
WAG0027	27(WWR)	30/10/2003	Geographe Bay	-
WAG0027	80(WWR)	20/11/2004	Geographe Bay	388
WA0227	323	29/03/2010	Perth Canyon	2343
WAG0033	33(WWR)	31/10/2003	Geographe Bay	-
WA0111	136	8/02/2004	Perth Canyon	1883
WA0111	315	4/04/2009	Perth Canyon	1983
WA0111	319	8/04/2009	Perth Canyon	1987
WA0031	29	4/03/2001	Perth Canyon	-
WAG0030	30(WWR)	31/10/2003	Geographe Bay	971
WA0205	292	6/04/2005	Portland	-
Not Assigned yet	76	13/03/2010	Bonney Upwelling	1803
WA0048	92	10/02/2002	Perth Canyon	-
Not Assigned yet	94	24/03/2010	Bonney Upwelling	2964
WAG143	144	19/12/2009	Geographe Bay	-
Not Assigned yet	75	13/03/2010	Bonney Upwelling	85

Abundance estimates

Not completed. A minimum of 3-year sampling was required to conduct the mark-recapture

analysis with the open population models. Unfortunately, only one year of funding was provided and an attempt to secure the additional 2 years of funding necessary for analysis was unsuccessful. A new grant application, including a power analysis, will be submitted in the next AMMC round, to request funding to continue this project. This study has been identified as a research priority at the Blue Whale Workshop held in Adelaide in July 2010.

3. Appropriateness

The appropriateness of the approaches used in the development and implementation of the Activity

The methods and approaches performed appropriately for carrying out the secondary objectives of the project. In particular, the new microsatellite genetic markers that were developed, optimised and utilised were successful in determining population and subspecies identity and detecting migrants and admixed individuals from the two Southern Hemisphere blue whale subspecies. The photo-identification methods utilised in the project are standard in blue and other whale studies. The teams involved in the photo-identification component of the project in the three different areas (Centre for Whale Research, Western Whale Research, and Blue Whale Study) are currently in the process of uploading their catalogues into the Southern Hemisphere Blue Whale Catalogue. This should speed up the process of matching between areas in Australia and others in the southern hemisphere. Between-year genetic recaptures, which were not found during initial photo-ID analysis, were confirmed by photo-ID based on the prior knowledge that these were genetic recaptures, emphasising the benefit of using both photo-ID and genetic methods in mark-recapture studies of blue whales. A mark-recapture photo-identification analysis of blue whales was previously conducted for Perth Canyon, demonstrating its feasibility as a method for estimating abundance of these whales. The funding of only one year for the project, without the additional funding of two years as originally requested in the project proposal, made it unfeasible for the project to complete its primary objective: estimating abundance of blue whales using mark-recapture, open population models. We are revising our sampling scheme and will be conducting a power analysis to estimate the effort necessary to obtain the appropriate amount of data for conducting the population modelling.

4. Effectiveness

The degree to which the Activity has effectively met its stated objectives

The project has effectively met its secondary objectives of genetic and photo-identification. It was not effective in its primary objective of estimating abundance due to lack of funding to complete the three years of sampling necessary and possibly small number of resightings. We will conduct a power analysis, and revise our sampling scheme accordingly, with the aim of providing evidence on the appropriateness of this method for estimating abundance of blue whales in Australian waters. This will be part of a new grant application to the AMMC.