Australian Marine Mammal Centre (AMMC)

Final Report

Season 2009/10

- **Title** - Population genetics and phylogeography of Australian snubfin and humpback dolphins: defining appropriate management units for conservation- Stage 1.
- **Chief Investigator** - Dr Guido J. Parra
- **Organisation** – Flinders University

Activity Period –31 January 2010-31 November 2010

Table of contents

1. Project progress and objectives achieved
2. Milestones and timeframes met
3. Delays affecting project
4. Budget
5. Current Balance of Account
6. Income and expenditure statement
7. Attachments

1. Project progress and objectives achieved

A clear summary of the work undertaken in the period to which the Report relates including the objectives achieved and an analysis of the effectiveness of this work

The objectives of this project have been achieved in full.

Objectives:

1. To investigate the spatial population genetic structure and rates of dispersal or gene flow among populations of snubfin and humpback dolphins at the state level in Queensland

   The work was completed successfully as planned (see Attachment 1). All Queensland samples available to 2009 have been analysed for microsatellites and mitochondrial DNA. Protocols for DNA extraction and amplification have been optimized. Further data analyses on population structure, migration rates and sex-biased dispersal using Bayesian clustering algorithms, assignment tests and bi-parentally inherited genetic markers are currently under way for peer review publication.

2. To initiate collection of biopsy samples of Australian snubfin and humpback dolphins in localities where samples are lacking: Northern Territory and Western Australia.

Biopsy sampling of *Sousa* and *Orcaella* was successfully carried out in Western

Australia and Northern Territory during April-October 2010. A total of 58 samples of humpback dolphins and 41 samples of snubfin dolphin were collected (See Table 1).

Detailed accounts of biopsy sampling activities in WA and NT are provided by chief investigators responsible for sampling in these areas (WA: Dr. Lars Bejder and Dr. Deborah Thiele and NT: Carol Palmer) in attachments 4-6 and

Table 1. Summary of biopsy samples of humpback and snubfin dolphins collected in Western Australia and Northern Territory during April-October 2010.

State	General Location	Species	
		Humpback dolphins	**Snubfin dolphins**
Western Australia	Exmouth	24	0
	Coral Bay	3	0
	Dampier	20	0
	Port Hedland	1	0
	Roebuck Bay	0	35
Northern Territory	Darwin Harbour	3	6
	Cobourg	7	0
Total		**58**	**41**

The inter-institutional and collaborative approach provided an unexpected amount of samples for most areas. Darting of snubfin and humpback dolphins is challenging and time consuming due to the inconspicuous nature of this species in comparison to other coastal dolphins (e.g. bottlenose dolphins). Thus our initial efforts in collecting samples across different areas in WA and NT have proved very successful. Genetic analyses of all samples collected are currently underway at Dr. Krützen's molecular laboratory at University of Zurich.

The sampling further substantiated the potential importance of some areas in WA for humpback dolphins (Exmouth, Coral Bay, Dampier,) and snubfin dolphins (Roebuck Bay). The project also confirmed the presence of both species of coastal dolphins in Darwin harbour and Cobourg.

The aim of this study was to take the first step towards the understanding of the population genetic structure and phylogeographic patterns of snubfin and humpback dolphins within Australian waters for conservation purposes. Understanding of population genetic structure and levels of gene flow of these potentially endemic and threatened species is paramount to their monitoring, management and conservation. The first stage in these process included the analysis of population structure at the state level in Queensland where a good coverage and sample size is already available.

Altogether, our data suggest that humpback and snubfin dolphin populations along the urban coast of Queensland are genetically differentiated into at least two to three distinct genetic clusters: Northern, central and south Queensland. Our results also highlight that further subdivisions within these clusters are evident for humpback dolphins. The low migration rates of dolphins between these major areas suggest that populations of snubfin and humpback dolphins from these three areas should be

considered as separate entities and considered independently for further actions towards their conservation and management.

Long-term standardized collection and analysis of biopsy samples from under represented areas across the dolphins range will be critical to determine the spatial population structure patterns of these coastal dolphins at a national scale. This will be particularly useful to guide future research effort on these species and to inform the planning and management of inshore waters in remote and urban regions of Australia where these species occur. Given the extent of snubfin and humpback dolphins range in Australia, gathering this data will require multidisciplinary research collaborations across the range of these species. We have demonstrated through our collaboration considerable potential to acquire the data required towards such purposes. Our vision is that phase two of this study (2011-2012) will provide the needed sample sizes from other areas across the range of both species in northern Australia to provide a clear picture of population genetic structure at a national scale.

2. Milestones and timeframes met

Identification of the Milestones and timeframes (and any performance indicators) met during the period to which the Report relates

We have met all milestones outlined in funding agreement

Milestone	Due Date	Actual Date
Signing of Agreement		
1st Progress Report submitted to and accepted by the Department including: 1) Preliminary screening and optimisation of both mtDNA and microsatellite markers 2) mtDNA and microsatellite analysis of Queensland samples 3) Scientific workshop 4) Full sharing of data and samples through a data sharing agreement and a centralised sample archive	12 Feb 2010	12 Feb 2010
Final report submitted to and accepted by the Department including detail outcomes of all of the work undertaken for the project:	22 Nov 2010	22 Nov 2010
1) Analysis of Queensland samples 2) Biopsy sampling in the Northern Territory and Western Australia	1) See detail report on activities in attachment 1. 2) see detailed report on activities in attachment 2 and 3	

3. Delays affecting project

A statement as to whether the timeframes for the Activity are being met and an explanation of any delays that have occurred, including the reasons for those delays and the action the Organisation proposes to take to address the delay and the expected effects (if any) the delay will have on the Activity (including subsequent Milestones and the overall completion of the Activity)

We consider that the project met its objectives in full with no major delays.

Attachment 1. *Please do not cite without authors permission.*

Genetic diversity and population structure of Australian Snubfin and Indo-Pacific humpback dolphins along the east coast of Queensland

Introduction

Australian snubfin (*Orcaella heinsohni*) and humpback dolphins (*Sousa chinensis*) are found in coastal waters of Queensland, Northern Territory and Western Australia. The Australian snubfin dolphin was recently described as a new species and it is the only cetacean endemic to Australian/Papua New Guinean waters (Beasley et al., 2005). The taxonomy of the humpback dolphin, Genus *Sousa*, however, remains complex and unresolved (Jefferson and Karczmarski, 2001; Jefferson and Waerebeek, 2004), although recent preliminary phylogenetic analyses of mitochondrial DNA sequences strongly suggest that Australian humpback dolphins likely represent a distinct species (Frère et al., 2008).

Estimates of population size in local areas along the Queensland coast indicate that populations of both species are notably small making them particularly vulnerable to human-induced disturbances on coastal ecosystems (Parra et al., 2006a). Moreover photo-identification data suggests moderate levels of site fidelity in both species making them potentially vulnerable to habitat degradation and loss given their restricted coastal distribution (Parra et al., 2006a). Both humpback and snubfin dolphins are likely to exist as metapopulations (small and partially or completely isolated populations). This makes them susceptible to extinction if rates of dispersal between populations are adversely affected (Hanski, 1998; Tilman et al., 1994). Extinction rates are further accelerated by loss of genetic variation in populations with abnormally low immigration and small population sizes (Bouzat, 2000; Bouzat et al., 1998; Forney and Gilpin, 1989; Frankham, 1995). Without a knowledge of the metapopulation structure and degree of dispersal and hence an understanding of how to manage the metapopulations, there are serious concerns about the conservation and long-term survival of these species in Australian waters. Despite conservation concerns and a recent increase in studies investigating the ecology of Australian snubfin and Indo-Pacific humpback dolphins (Parra, 2006; Parra, 2007; Parra et al., 2002; Parra and Corkeron, 2001; Parra et al., 2004; Parra et al., 2006a; Parra et al., 2006b), their genetic variability and levels of gene flow among populations across their range remains unknown.

Demographic, environmental, and genetic factors contribute to population or species viability. Maintaining adequate levels of genetic diversity, within and among populations, is one critical aspect to consider for maintaining population viability and one of the main principles underlying the management of threatened species. Genetic variation of a single species should be analysed starting at the highest level, *i.e.*, the variation among populations, which include numbers of subspecies, interpopulation genetic structure of marker loci, and demographic factors such as the range of environments in which different populations are found, since they may reflect differences of adaptation. Quantification of genetic variability and gene flow, or lack thereof, among wild populations of snubfin and humpback dolphins will provide an assessment of how populations are spatially structured and the degree of dispersal across their range. This knowledge will contribute to define 1) appropriate

geographical scales for management of populations and 2) populations or genetic groupings that should be managed separately (e.g. Evolutionary Significant Units and Management Units) to best maintain evolutionary processes and adaptive diversity across the geographic range of the species (Moritz, 1994; Palsboll et al., 2007). Such information can contribute significantly to the development of a decision process for choosing targets for protection, achieving conservation objectives and to inform the development of marine management areas for snubfin and humpback dolphins in Northern Australia. For example, if genetic homogeneity prevails across the range of Australian snubfin and/or humpback dolphins, from a genetic perspective, management actions should focus on the entire population of both species at a national level. On the other hand, if there is strong evidence of genetic divergence across populations, management actions should focus on the distinct populations or systems of several such populations. Determining the exact management strategy to follow based on information on the genetic population structure is not straightforward (Crandall et al., 2000; Fraser and Bernatchez, 2001); however it represents the first step towards defining targets for protection (Wood and Gross, 2008).

The aim of this study is to understand the population genetic structure and phylogeographic patterns of snubfin and humpback dolphins within Australian waters for conservation purposes. Here we present preliminary results for the analysis of population structure at the state level in Queensland where a good coverage and sample size is already available. Our vision is that this study in the long-term, coupled with concurrent undergoing studies at a local scale on the ecology of snubfin and humpback dolphins, will provide the best scientific information about the biological risk faced by these species and assist decision makers in combining this with other social, economic and political information towards the protection of Australian snubfin and humpback dolphins at a national scale in a context of adaptive management.

Methods

Sample collection

A total of 82 samples of Australian snubfin and 126 samples of humpback dolphins were collected from different localities along the east coast of Queensland (Table 1). Samples consisted of skin tissue (Humpback = 114, Snubfin = 48, Table 1) from free-ranging or stranded animals, and bone or teeth from stranded animals held in museums (Humpback = 12, Snubfin = 34, Table 1). Biopsy samples (skin tissue) were used for microsatellite genotyping and mithochondrial DNA (mtDNA) sequencing. Due to the low concentration of nuclear DNA in bone or teeth samples from museums, these samples were only used for mtDNA sequencing.

Skin samples of free-ranging animals were obtained using a biopsy system designed for small cetaceans (PAXARMS, Krützen et al., 2002). Biopsies at each sampling site were obtained from individuals from multiple dolphin groups, including solitary individuals. No samples were collected from dependent calves. All biopsy samples were preserved in a salt-saturated solution of 20% dimethyl sulphoxide for later analysis (Amos and Hoelzel, 1991). Bone samples from the skull of museum specimens were collected using a hand-held drill with a 1.0–1.5 mm drill bit following protocols in Pichler *et al.* (Pichler et al., 2001).

Table 1. Total number of Australian snubfin and humpback dolphin samples across different regions along the east coast of Queensland.

	Humpback dolphins		Snubfin dolphins	
Region	Biopsy (Skin tissue)	Museum (Bone or teeth)	Biopsy (Skin tissue)	Museum (Bone or teeth)
Hinchinbrook (Hinc)	8		2	
Townsville (Town)	17	12	37	34
Keppel Bay (Kepp)	24		9	
Gladstone (Glad)	15			
Northern Great Sandy Strait (NGSS)	12			
Southern Great Sandy Strait (SGSS)	23			
Moreton Bay (More)	15			
Total	114	12	48	34

Molecular analysis

DNA extraction and sexing

Total genomic and mitochondrial DNA from biopsy samples was isolated using the QIAGEN DNeasy Blood and Tissue Kit according to manufacturer's recommendations. DNA from bones or teeth was isolated following specific protocols of the EZ1 DNA Investigator Kit and a Biorobot from QIAGEN.

The sex of the animals biopsied was determined by amplification of the genes ZFX and SRY through the polymerase chain reaction (PCR) (Gilson et al., 1998) as described in Be´rube´ and Palsbøll (Berube and Palsboll, 1996). PCR reactions consisted of: 20 ng of genomic DNA in a 20 μl reaction containing 10 mM dNTPs, 5U/μl *Taq* DNA polymerase, 25 mM MgCl2 and 0.1 µm of each primer. The PCR cycling profile consisted of 94 ^{o}C for 60 sec followed by 40 cycles of 94 ^{o}C for 30sec, 58 ^{o}C for 30sec, 72 ^{o}C for 60 sec and 72 ^{o}C for 10 sec. The sex of museum specimens was taken from specimen's records.

Microsatellite genotyping

Biopsy samples were genotyped at 27 polymorphic cetacean microsatellite loci: D22 (Shinohara et al., 1997), EV37 (Valsecchi & Amos 1996), KWM12(Hoelzel et al., 1998), MK3, MK5, MK6, MK8, MK9 (Krützen et al., 2001), D8, E12, F10, TUR4_105, TUR4_108, TUR4_111, TUR4_117, TUR4_128, TUR4_132, TUR4_138, TUR4_141, TUR4_142, TUR4_153, TUR4_162, TUR4_66, TUR4_80, TUR4_87, TUR4_91, and TUR4_98 (Nater and Krützen, 2009). We had consistent problems amplifying locus TUR4_132 for humpback dolphins and F10 for snubfin dolphins thus these were excluded from respective multiplexes. PCRs contained 20 ng template DNA, 5 µL 2× Multiplex PCR Master Mix (QIAGEN, containing HotStar*Taq* DNA Polymerase, dNTPs and 3 mm MgCl2 final concentration), 0.1 µm of each primer and double-distilled water to 10-µL volume. The following PCR profile was used for amplification: initial denaturation at 95 °C for 15 min, 25 cycles of 30 sec at 95 °C, 90 sec at 60 °C and 45 sec at 71 °C, followed by a final extension step of 30 min at 60 °C. One microlitre of the PCR product was diluted in 50 µL of double-distilled water and added to 10 µL Hi-Di formamide containing 0.07 µL GeneScan 500 LIZ size standard (Applied Biosystems), followed by denaturing for

3min at 95 °C. Samples were run on an ABI PRISM 3730 DNA Analyser and analysed with Gene-Mapper version 4.0 software (Applied Biosystems).

Mithochondrial DNA (mtDNA) screening and sequencing:

The mitochondrial DNA control region was amplified using PCR and primers dlp1.5 and dlp3R (Baker *et al.* 1993). PCR conditions were as follows: initial denaturation step at 94°C for 1 min, followed by a touch-down PCR with 9 cycles, decreasing the annealing temperature by 1°C per cycle. Denaturation was at 94°C (30 s), annealing at 63 to 55°C (1 min) and extension at 72°C (1 min). A cycle of 94°C (30 s), 52°C (30 s) and 72°C (1 min) was then repeated 29 times, followed by a final extension of 72°C for 10 min. PCR products were cleaned using QIAquick PCR purification kit (QIAGEN) according to the manufacturer's instructions. PCR products were then amplified with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's specifications, and sequenced in an ABI PRISM 3730 DNA Analyser. Sequences were edited by eye using SEQUENCING ANALYSIS software, version 5.2 (Applied Biosystems).

Data analyses

Identification of replicate samples and microsatellite scoring errors

Individual snubfin and humpback dolphins can be identified reliably through photo-identification (Parra et al., 2006a). During biopsy sampling of free-ranging animals we made efforts to obtain good quality photographs of the individuals been biopsied to minimize duplicate samples, but this was not always possible. Animals sampled more than once were identified using the Excel Add-Inn MSTOOLS Ver. 3.1 (Park, 2001) and removed from the data set. The microsatellite data set was then screened for genotyping errors with the software MICROCHECKER Ver. 2.2.3(Van Ooseterhaut et al., 2004).

Genetic variability within populations

To asses genetic diversity we provisionally subdivided samples of humpback dolphins into 7 different groups according to the sampling locality (Table 1). Samples of snubfin dolphins were divided into 3 groups (Table 1). We based our assignments on photo-identification data available for these populations and distance between locations. Photo-identification studies in all areas sampled have yielded few or no matches, indicating that animals rarely move between these areas.

For the microsattelite data, Observed (H_O) and expected (H_e) heterozygosity were calculated using ARLEQUIN ver 3.1.1(Excoffier et al., 2005). Allelic richness was calculated as described by El Mousadik and Petit (El Mousadik and Petit, 1996) using FSTAT version 2.9.3 (Goudet, 2002). Deviation from the Hardy-Weinberg Equilibrium (HWE) and tests for linkage disequilibrium for each locus at each locality, was assessed using the Fishers exact test and significance levels were evaluated using a Markov-chain randomization procedure (Guo and Thompson, 1992; Raymond and Rousset, 1995) in ARLEQUIN ver 3.1 (Excoffier et al., 2005). Additionally, we used MICROCHECKER 2.2.3 (Van Ooseterhaut et al., 2004) to assess the potential presence of null alleles and large allele dropout.

Alignment of the mtDNA sequences was done using the ClustalW algorithm (Thompson et al., 1994) implemented in the program Geneious (Drummond et al., 2009). Nucleotide diversity (π) and haplotype diversity (h) for the mtDNA data were estimated for each population using the program ARLEQUIN.

Genetic differentiation among populations

Genetic divergence among the different regions sampled was estimated using FST statistics (Weir & Cockerham, 1984), which assumes an infinite allele model of mutation, implemented in the program FSTAT. If the presence of null alleles was likely, we calculated unbiased F_{ST} estimates accounting for null alleles following the *ENA* correction method in FreeNa (Chapuis and Estoup, 2007). We compared F_{st} estimates of population differentiation using both the original and the corrected datasets. Significance levels for all multiple comparisons in this and all other tests described above were Bonferroni corrected.

To test for isolation by distance (Slatkin, 1993) we use simple Mantel test to evaluate correlations between genetic and geographical distances of sampled individuals and spatial autocorrelation analyses implemented in the software package ALLELES IN SPACE (AIS) (Miller, 2005). The genetic distance calculated in AIS is identical to that used by Nei *et. al.* (Nei et al., 1983)for population frequency data, but is instead applied to pairs of individuals rather than pairs of populations. No a priori assumptions regarding the geographic delineation of populations are necessary. Statistical significance of Mantel Tests was assessed using 5,000 randomisations. The measure of spatial autocorrelation used in AIS for analysis (A_y) is quantified as the average genetic distance between pairs of individuals that fell into a distance class y. Analyses were performed over 10 distance classes and a randomization procedure consisting of 5,000 replicates was used to identify distance classes where average genetic distances were significantly larger or smaller than random expectations.

To further assess the presence of population structure and the number of putative populations (K) that best explain the patterns of genetic variability observed we used the Bayesian clustering approach implemented in STRUCTURE (Pritchard et al., 2000) for K = 1–10. The posterior probability of the data [Ln P(D)] for each value of K was inferred from multilocus genotypes 10 times with 100,000 Markov chain Monte Carlo (MCMC) repetitions each and a burn-in period set at 10,000. Given the geographical extent of the sampling locations and presumably moderate levels of gene flow, we assumed populations were admixed, that allele frequencies were independent between populations, and ran the model with and without prior information on sampling location (Falush et al., 2003). We used the ad hoc statistic ΔK to detect the best estimate of the real number of putative populations (Evanno et al., 2005).

Sex-biased dispersal

We tested for sex bias in dispersal in FSTAT using microsatellites data and comparing four different statistics: (1) sex-specific FST; where higher FST values are expected for the philopatric sex than the more dispersing sex; 2) FIS; where members of the dispersing sex should display higher FIS values than the more philopatric sex; 3) mean of assignment index (mAIc), where the average index for the sex that disperses most is expected to be lower than that for the more philopatric sex; and 4) the variance of the assignment index (vAIC), where individuals from the dispersing sex are expected to have higher vAIC than the philopatric sex (Goudet et al., 2002). Statistical significance was assessed through 10,000 randomizations for each test.

Migration rates

We used the Bayesian multilocus genotyping approach implemented in the program BAYESASS Ver. 1.3 (Wilson and Rannala, 2003) to estimate recent rates of migration between dolphins from the different populations sampled. This method allows for genotype frequencies to deviate from Hardy-Weinberg equilibrium and accounts for unequal migration rates. For estimating posterior probability distributions of parameters, the MCMC was run for a total of 5×10^6 iterations, with the first 10^6 iterations acting as burn-in to allow the chain to reach stationarity. Samples were collected every 2000 iterations to infer posterior probability distributions.

Results

We identified 14 biopsy samples from humpback dolphins and 7 of snubfin dolphins that showed either identical microsatellite genotypes or higher than 95% matching alleles at all loci with another sample. These samples were considered duplicates and excluded from further analyses. Two biopsy samples from humpback and three from snubfin dolphins failed to amplify for several loci and were dropped from analysis, leaving a total of 98 and 38 samples, respectively. None of the loci showed evidence of genotyping errors for either species.

Humpback dolphins

Genetic variability within populations

Out of the 26 loci we genotyped for humpback dolphins 6 were monomorphic (D22, D8, F10, Tur 4_87, Tur4_108, and Tur4_111) for all populations. The mean number of alleles observed per locus, across the remaining 20 loci ranged from 1.95 in SGSST to 2.70 in Townsville (Table 2). Mean Allelic richness was similar across all population ranging from 1.8 in SGSS to 2.51 in Townsville (Table 2). Observed and expected heterozygozities were similar for all groups with an overall average of 0.32 and 0.36, respectively (Table 2).

Table 2. Measures (Mean ± SE) of genetic variability in humpback dolphins from east coast of Queensland based for 20 microsatellite loci and mitochondrial DNA (mtDNA) control region sequences. n = sample size; AD = Allelic diversity, AR = Allelic Richnes, Ho = observed heterozygosity, He = expected heterozygozyty, NH = number of haplotypes; h = haplotypic diversity; and π = nucleotide diversity.

	Microsatellites					**mtDNA**			
Region	**n**	**AD**	**AR**	**Ho**	**He**	**n**	**NH**	**h**	**π**
Hinchinbrook	8	2.35 ± 0.20	2.32 ± 0.19	0.40 ± 0.06	0.40 ± 0.05	8	2	1.0 ± 0.022	0.004 ± 0.001
Townsville	13	2.70 ± 0.24	2.51 ± 0.20	0.39 ± 0.06	0.44 ± 0.05	20	3	1.0 ± 0.004	0.002 ± 0.000
Keppel Bay	21	2.40 ± 0.22	2.13 ± 0.20	0.37 ± 0.05	0.37 ± 0.05	20	3	1.0 ± 0.004	0.007 ± 0.001
Gladstone	13	2.35 ± 0.21	2.16 ± 0.18	0.32 ± 0.05	0.34 ± 0.05	13	3	1.0 ± 0.008	0.008 ± 0.001
NGSS	12	2.35 ± 0.20	2.17 ± 0.15	0.38 ± 0.05	0.35 ± 0.04	12	1	1.0 ± 0.010	0.000 ± 0.000
SGSS	18	1.95 ± 0.14	1.80 ± 0.12	0.28 ± 0.05	0.27 ± 0.05	18	1	1.0 ± 0.004	0.000 ± 0.000
Moreton Bay	13	2.25 ± 0.16	2.13 ± 0.14	0.33 ± 0.05	0.37 ± 0.05	12	1	1.0 ± 0.010	0.000 ± 0.000

We observed significant departures from HWE in Townsville for locus EV37 (P = 0.01) and in Moreton Bay at locus MK5 (P = 0.02) after Bonferroni correction. There was no evidence of significant linkage disequilibrium between any pair of loci. Potential null alleles were detected with MICROCHECKER at Townsville (for locus EV37, MK3, TUR4_105, and TUR4_117), Keppel Bay (for locus TUR4_141), and Moreton Bay (for locus MK5).

A sequence fragment of 428 bp of the mtDNA control region was successfully aligned for 103 samples of humpback dolphins across all localities. We found a total of 5 unique haplotypes characterised by 11 polymorphic sites (Table 3). Haplotype and nucleotide diversity was similar for all localities (Table 2). The most common haplotype (E, 58% of all individuals sampled) was found in Keppel Bay, Gladstone, NGSS, SGSS and Moreton Bay. All individuals from NGSS, SGSS and Moreton Bay were of haplotype E. The second most common haplotype was C (22%) which was found in individuals from Hinchinbrook, Townsville and Keppel Bay (Table 3).

Genetic differentiation among populations

Given the potential presence of null alleles in some localities we used the program FreeNA to calculate a corrected dataset for these loci and compare with original dataset. Global estimates of population differentiation across localities were similar (t-test =-1.11, P = 0.28) between the original data (F_{st} = 0.139, 95% CI = 0.092 to 0.182) and the corrected dataset (F_{stNA} = 0.137, 95% CI = 0.093 to 0.181) indicating the presence of null alleles appear to have no significant effect. Therefore the results presented here correspond to the original dataset unless specified otherwise.

Significant population differentiation was detected between all sampling regions for the microsatellite data (Table 4). Comparisons based on the mtDNA data set indicated significant differentiation between populations in the north (Hinchinbrook and Townsville) and all other localities further south. However, no genetic differentiation was detected between Hinchinbrook and Townsville. Keppel Bay and Gladstone showed significant differentiation with SGSS but not with NGSS and Moreton Bay (Table 4).

Table 3. Polymorphic sites and distribution of mtDNA control region haplotypes for humpback dolphins across 7 localities in east coast of Queensland.

	Position											Population*							
	18	73	86	190	250	267	286	310	311	342	377	Hinc	Town	Kepp	Glad	NGSS	SGSS	More	Total
HapA	C	A	G	C	G	C	C	G	T	T	C	0	0	2	5	0	0	0	7
HapB	C	A	G	C	G	T	C	A	C	C	A	2	1	0	1	0	0	0	4
HapC	C	A	G	C	G	T	C	G	T	T	C	6	10	7	0	0	0	0	23
HapD	C	G	G	C	G	T	C	G	T	T	C	0	9	0	0	0	0	0	9
HapE	T	A	A	T	A	C	T	G	T	T	C	0	0	11	7	12	18	12	60
											Total	8	20	20	13	12	18	12	103

*Hinchinbrook (Hinc); Townsville (Town); Keppel Bay (Kepp); Gladstone (Glad); Northern Great Sandy Strait (NGSS); Southern Great Sandy Strait (SGSS) and Moreton Bay (More)

Table 4. Pairwise F_{st} values between humpback dolphin sampling regions calculated with microsatellite (below diagonal) and mtDNA data (above diagonal). Significant values (*P < 0.05. and **P < 0.01) after Bonferroni corrections are marked with an asterisk.

	Hinchinbrook	**Townsville**	**Keppel Bay**	**Gladstone**	**NGSS**	**SGSS**	**Moreton Bay**
Hinchinbrook		0.188	0.418*	0.431*	0.903**	0.925**	0.903**
Townsville	0.072*		0.506**	0.556**	0.914**	0.927**	0.914**
Keppel Bay	0.158**	0.126**		-0.034	0.340	0.392*	0.340
Gladstone	0.192**	0.151**	0.041*		0.345	0.411*	0.345
NGSS	0.137**	0.103**	0.141**	0.112**		0.000	0.000
SGSS	0.250**	0.211**	0.146**	0.145**	0.074**		0.000
Moreton Bay	0.167**	0.105**	0.170**	0.161**	0.114**	0.185**	

Mantel tests of isolation by distance indicated a small, but significant correlation between genetic and geographic distances in humpback dolphins (r = 0.38, P = 0.0002, Fig. 1). Similarly, spatial autocorrelations illustrated that pairwise genetic distances were significantly smaller than average over shorter distances (up to 380 Km) and were significantly larger than random expectations as geographic distances increased (Fig. 1). Results of these analyses suggest that the extent of spatial genetic structure in humpback dolphins occurs in the order of approximately 380 km.

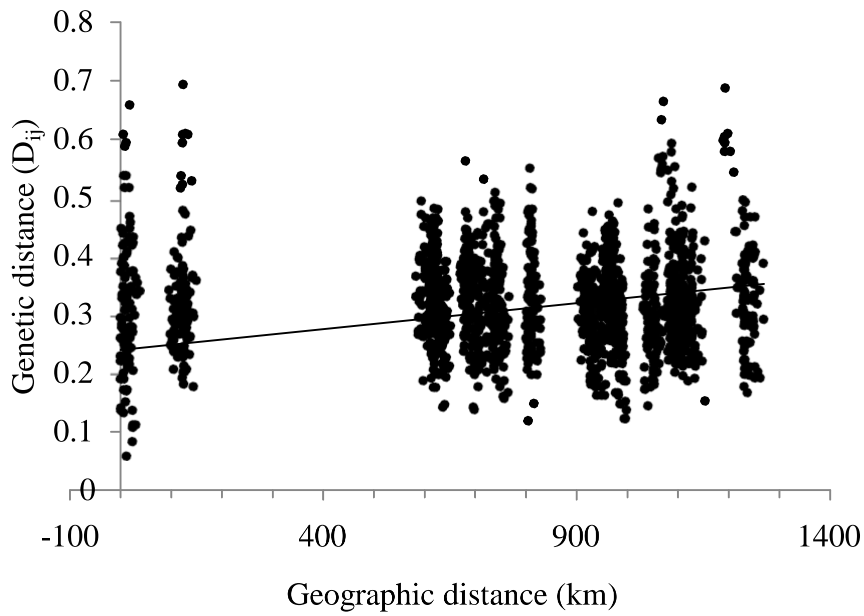
Genetic clustering analysis in STRUCTURE for models with and without prior information on sampling location revealed that the posterior probability [Ln P(D)] was highest at K = 4 while the ad hoc statistic ΔK was highest at K = 3 (Fig. 2). Inspection of individual assignment probabilities indicates the most likely number of distinct genetic populations appears to be three (Fig. 3). At K = 3, most individuals where strongly assigned to one of three clusters (Fig. 3). The membership proportion (*Q*) of Hinchinbrook and Townsville samples to cluster (1) was high, with *Q* = 0.89 and *Q* = 0.73 respectively. Similarly samples from Keppel bay and Gladstone were assigned with high proportion to cluster 2 (Keppel Bay, *Q* = 0.92; Gladstone, *Q* = 0.79), and samples from NGSS, SGSS and Moreton Bay to cluster 3 (NGSS, *Q* = 0.78; SGSS, *Q* = 0.83; Moreton Bay, *Q* = 0.79).

Sex-biased dispersal

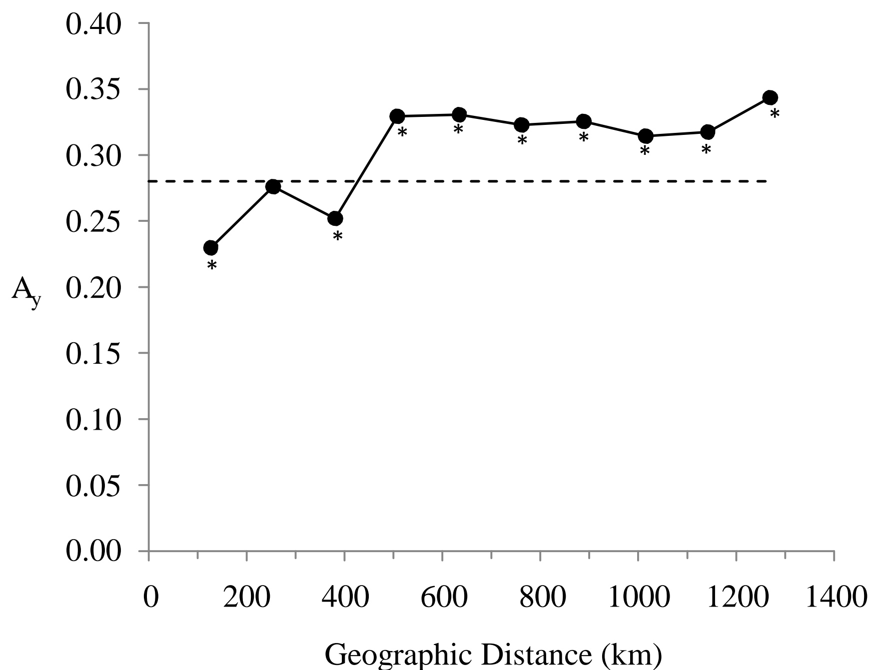
In total 56 female and 39 male humpback dolphins were available for analysis of sex biased dispersal. There was no indication of significant sex-biased dispersal based on the analyses conducted in FSTAT. FST and FIS values were similar for females

and males (FST for females = 0.15, males = 0.13; P = 0.43; FIS for females = 0.04, males = 0.01, P= 0.67). Differences between the mean and the variance

of the Assignment Index for females and males were also not significant (mAIc for females = 0.38, males = -0.54, P = 0.31; vAIC for females = 9.26, males = 24.60, P = 0.08).

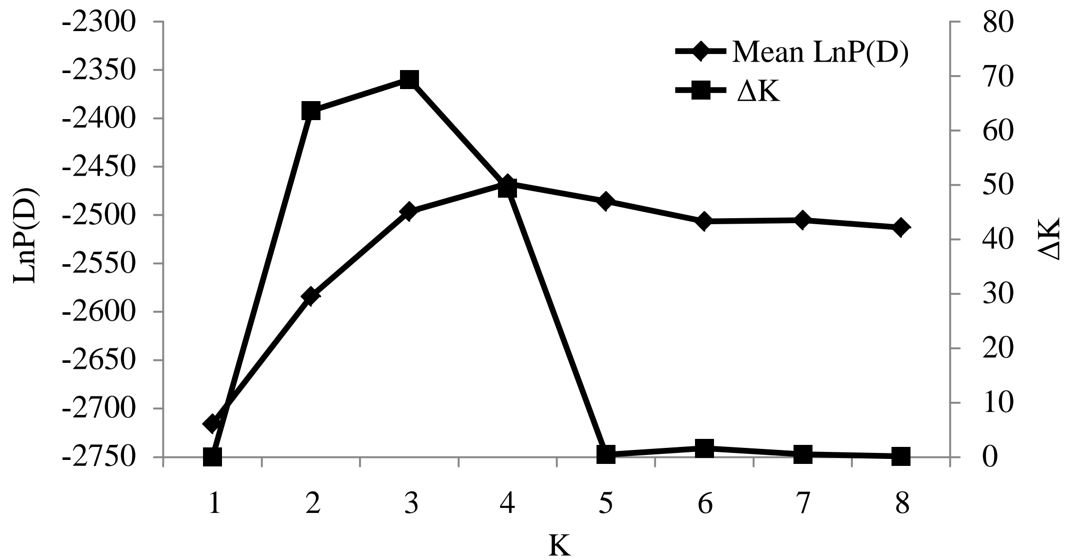


A)

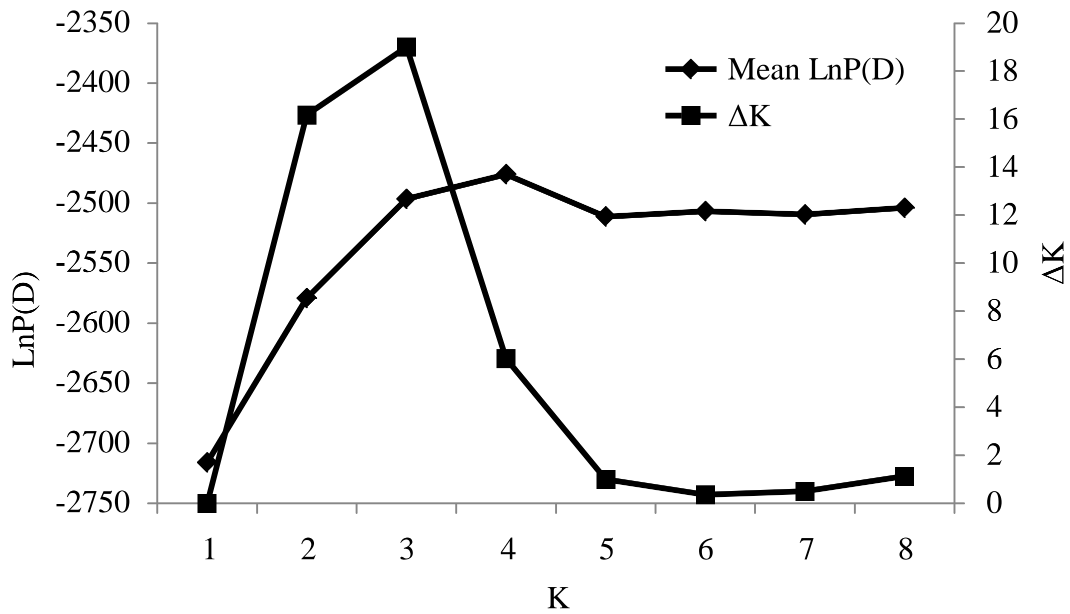


B)

Figure 1. Results of Mantel Test (A) and spatial autocorrelation analysis (B) of humpback dolphins along the Queensland coastline. Spatial autocorrelation analyses were performed using 10 distinct geographic distance classes. A_y quantifies the average pairwise genetic distances between samples that fall within the boundaries specified for distance class y. Horizontal lines indicate the average value of A_y for the data set. Distance classes in spatial autocorrelations that showed significantly larger or smaller values at the $\alpha = 0.05$ level than average are marked with asterisks.

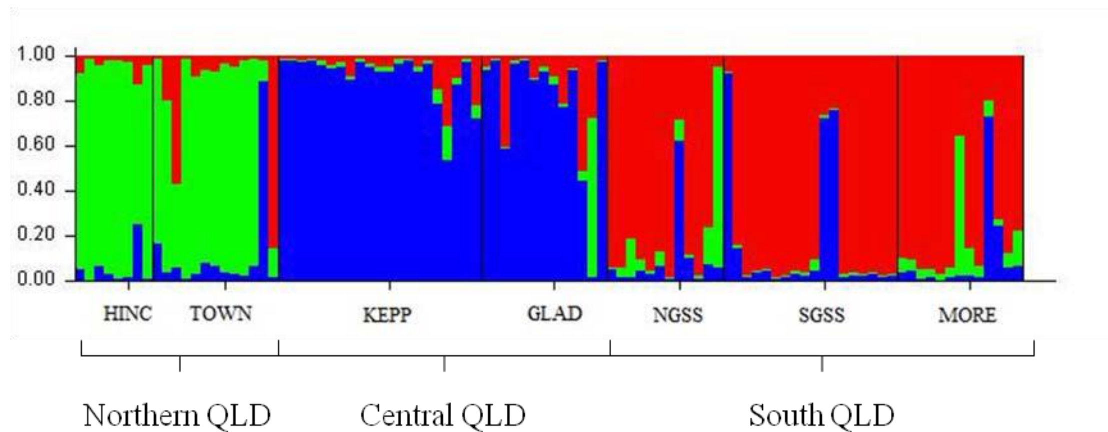


A)

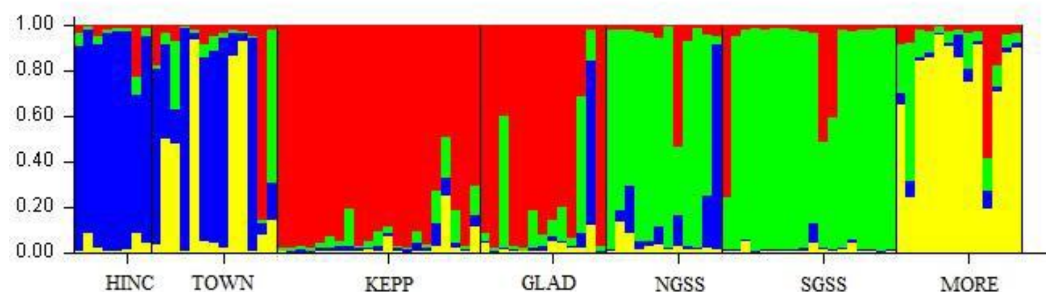


B)

Figure 2. STRUCTURE output of posterior probabilities [Ln P(D)] and ΔK (a measure of the second order rate of change in the likelihood of *K*) of humpback dolphins data for each value of K (the number of putative populations) under models without (A) and with (B) prior information on sampling location.



A)



B)

Figure 3. Summary plots of genetic clustering analysis in STRUCTURE for humpback dolphins sampled in seven localities (distinguished by black lines) along the east coast of Queensland. Each column represents one individual and colours correspond to the percentage of assignment to each cluster A) K = 3, B) K =4. The most likely number of genetic clusters in the data set was identified as three: Northern Queensland. Central Queensland and South Queensland. Sampling sites within these three clusters are on the X-axis organized from north (left) to south (right): HINC = Hinchinbrook; TOWN = Townsville; KEPP = Keppel Bay; GLAD = Gladstone; NGSS = Northern Great Sandy Strait; SGSS = Souther Great Sandy Strait; and MORE = Moreton Bay.

Migration rates

Simulations in BAYEASS show that in instances where there is no information in the data, the mean and 95% confidence interval for the non-migration rates of humpback dolphins are 0.833 (0.675-0.992) and 0.0277 (0- 0.144) the migration rates. Confidence intervals obtained from the data set were considerably smaller than those obtained randomly (Table 5), suggesting that sufficient information was available to reliably estimate migration rates. Hinchinbrook, Gladstone, SGSS, and Moreton bay had a high proportion of individuals identified as non-migrant (94–98%) while Townsville, Keppel Bay, and NGSS locations appearing more admixed with only a 68-71% proportion of nonmigrants (Table 5). Overall, migration rates were very low, with only moderate migration estimated from Townsville to Hinchinbrook (m = 0.127, 95% CI = 0.031-0.234), from Keppel Bay to Gladstone (m = 0.285, 95% CI = 0-0.225) and from NGSS to SGSS (m = 0.227, 95% CI= 0.124-0.309).

Table 5. Mean (95% CI) posterior distributions for migration rates among humpback dolphins in Queensland calculated with the program BAYESASS. Values along the diagonal (bold) are the proportion of individuals each generation that are not migrants. Simulations show that in instances where there is no information in the data, the mean and 95% confidence interval for the non-migration rates are 0.833 (0.675-0.992) and for the migration rate are 0.0277 (0-0.144)

Migration rate	To						
From	Hinchinbrook	Townsville	Keppel Bay	Gladstone	NGSS	SGSS	Moreton Bay
Hinchinbrook	**0.952 (0.838-0.999)**	0.007 (0- 0.046)	0.007 (0-0.43)	0.011 (0-0.073)	0.008 (0-0.068)	0.008 (0-0.60)	0.007 (0-0.48)
Townsville	0.127 (0.031-0.234)	**0.712 (0.673-0.780)**	0.012 (0-0.059)	0.014 (00.070)	0.017 (0-0074)	0.023 (0-0.088)	0.095 (0.010-0.222)
Keppel Bay	0.007 (0-0.036)	0.006 (0-0.032)	**0.682 (0.667-0.719**	0.285 (0-0.225)	0.006 (0-0.031)	0.007 (0-0.045)	0.007 (0-0.035)
Gladstone	0.007 (0-0.041)	0.006 (0-0.039	0.006 (0-0.039)	**0.940 (0.846-0.992)**	0.018 (0-0.071)	0.015 (0-0.086)	0.007 (0-0.043)
NGSS	0.016 (0-0.087)	0.011 (0-0.057)	0.011 (0-0.056)	0.028 (0-0.106)	**0.694 (0.668-0.769)**	0.227 (0.124-0.309)	0.013 (0-0.070)
SGSS	0.003 (0-0.019)	0.003 (0-0.023)	0.003 (0-0.022)	0.005 (0-0.034)	0.003 (0-0.022)	**0.980 (O.933-1)**	0.003 (0-0.022)
Moreton Bay	0.006 (0-0.038)	0.005 (0-0.034)	0.005 (0-0.033)	0.007 (0-0.043)	0.008 (0-0.053)	0.012 (0-0.069)	**0.958 (0.868-0.999)**

Snubfin dolphins

Genetic variability within populations

A total of 13 loci out of the 26 loci we genotyped for snubfin dolphins were monomorphic (D22, D8, Mk5, Mk9, Tur4_66, Tur4_91, Tur4_108, Tur4_111, Tur 4_128, Tur4_132, Tur4_138, and Tur4_162,) for all populations. The mean number of alleles observed per locus, across the remaining 13 loci varied across localities (range =1.92 to 3.62, Table 6). Mean allelic richness was similar across all populations ranging from 1.71 in Keppel Bay to 1.92 in Hinchinbrook (Table 6). Observed and expected heterozygozities were similar across Townsville and Keppel Bay (Table 6).

Significant departures from HWE were only detected at one locus (EV37, P = 0.01) in the Townsville locality. There was no evidence of significant linkage disequilibrium between any pair of loci. Evidence of null alleles was detected with MICROCHECKER for locus EV37 for Townsville.

We found a total of 12 unique haplotypes characterised by 37 polymorphic sites over a sequence fragment of 448 bp of the mtDNA control region (Table 7). Haplotype and nucleotide diversity was higher in Hinchinbrook and Townville than Keppel Bay, while nucleotide diversity was highest in Townsville (Table 6). The most common haplotypes found were haplotype C (22%) and J (22%) which were only found in Townsville (Table 6).

Genetic differentiation among populations

Global estimates of population differentiation across localities were similar (t-test =-1.11, P = 0.28) between the original data (F_{st} = 0.159, 95% CI = 0.002 to 0.385) and the corrected dataset accounting for the presence of null alleles (F_{stNA} = 0.150, 95% CI = 0.007 to 0.354) indicating the presence of null alleles appear to have no significant effect. Therefore the results presented here correspond to the original dataset unless specified otherwise.

The microsatellite data showed significant population differentiation between Hinchinbrook and Keppel Bay, and between Townsville and Keppel Bay. No population differentiation was detected between Hinchinbrook and Townsville (Table 8). Comparisons based on the mtDNA data set indicated no significant differentiation between localities (Table 8).

Tests for associations between genetic and geographic distances indicated that geographic distance plays a role in the distribution of genetic variation among populations of snubfin dolphins (r = 0.5, P = 0.0002, Fig. 4) The results from the spatial autocorrelation analysis showed that individuals from the same sampling locations (0 km distance class) and those separated up to 116 km were significantly more similar than expected from random (Fig. 4). At distances > 600km populations are genetically less similar than expected from random.

Table 6. Measures (Mean ± SE) of genetic variability in snubfin dolphins from east coast of Queensland based for 13 microsatellite loci and mitochondrial DNA (mtDNA) control region sequences. n = sample size; AD = Allelic diversity, AR = Allelic Richnes, H_o = observed heterozygosity, H_e = expected heterozygozyty, NH = number of haplotypes; h = haplotypic diversity; and π = nucleotide diversity.

	Microsatellites					**mtDNA**			
Region	**n**	**AD**	**AR**	**H_o**	**H_e**	**n**	**NH**	**h**	**π**
Hinchinbrook	8	1.92 ± 0.21	1.92 ± 0.21	0.54 ± 0.12	1.0 ± 0.09	2	2	1.000 ± 0.354	0.004 ± 0.005
Townsville	28	3.62 ± 0.31	1.81 ± 0.14	0.37 ± 0.06	0.39 ± 0.06	61	10	0.844 ± 0.003	0.007 ± 0.004
Keppel Bay	8	2.23 ± 0.30	1.71 ± 0.18	0.35 ± 0.08	0.34 ± 0.08	9	2	0.500 ± 0.043	0.004 ± 0.003

Table 7. Polymorphic sites (a) and distribution (b) of mtDNA control region haplotypes for snubfin dolphins across 3 localities along the east coast of Queensland.

	Position																																				**Population***			
	19	**33**	**37**	**41**	**42**	**71**	**75**	**77**	**83**	**90**	**94**	**101**	**108**	**111**	**130**	**131**	**175**	**185**	**249**	**254**	**259**	**263**	**264**	**275**	**284**	**307**	**338**	**351**	**372**	**373**	**374**	**390**	**391**	**392**	**434**	**437**	**438**	**Hinc**	**Town**	**Kepp**	**Total**
HapA	A	A	G	T	T	T	T	T	C	G	G	T	T	C	C	A	T	C	C	T	C	C	C	C	G	T	T	T	C	C	T	T	A	A	C	C	T	1	0	0	1
HapB	G	G	A	C	C	C	C	C	T	A	A	C	C	C	T	G	G	T	A	A	T	T	T	C	A	T	C	C	C	C	C	C	G	G	C	T	C	1	8	0	9
HapC	G	G	A	C	C	C	C	C	T	A	A	T	C	C	T	G	G	T	A	A	T	T	T	C	A	T	C	C	C	C	C	C	G	G	C	T	C	0	16	0	16
HapD	G	G	A	C	C	C	C	C	T	A	A	T	C	C	T	G	G	T	A	A	T	T	T	C	A	T	T	C	C	C	C	C	G	G	C	T	C	0	1	0	1
HapE	G	G	A	C	C	C	C	C	T	A	A	T	C	C	T	G	G	T	A	A	T	T	T	T	G	T	C	C	C	C	C	C	G	G	C	T	C	0	3	0	3
HapF	G	G	A	C	C	C	C	C	T	A	A	T	C	C	T	G	T	T	A	A	T	T	T	C	A	C	C	C	C	C	C	C	G	G	C	T	C	0	1	0	1
HapG	G	G	A	C	C	C	C	C	T	A	A	T	C	C	T	G	T	T	A	A	T	T	T	C	A	C	C	C	T	C	T	T	A	A	T	T	C	0	1	0	1
HapH	G	G	A	C	C	C	C	C	T	A	A	T	C	T	T	G	G	C	A	A	T	T	T	C	A	T	C	C	C	C	C	C	G	G	C	T	C	0	6	0	6
HapI	G	G	A	C	C	C	C	C	T	A	A	T	C	T	T	G	G	T	A	A	T	T	T	C	A	T	C	C	C	C	C	C	G	G	C	T	C	0	3	0	3
HapJ	G	G	A	C	C	C	C	C	T	A	A	T	C	T	T	G	G	T	A	A	T	T	T	C	A	T	C	C	C	T	C	C	G	G	C	T	C	0	16	0	16
HapK	G	G	A	C	C	C	C	C	T	A	A	T	C	T	T	G	G	T	A	A	T	T	T	C	A	T	C	C	C	T	C	C	G	G	T	T	C	0	6	8	14
HapL	G	G	A	C	C	C	C	C	T	A	A	T	C	T	T	G	T	T	A	A	T	T	T	C	A	T	C	C	C	T	C	C	G	G	T	T	C	0	0	1	1
																																					Total	2	61	9	72

*Hinchinbrook (Hinc); Townsville (Town); and Keppel Bay (Kepp);

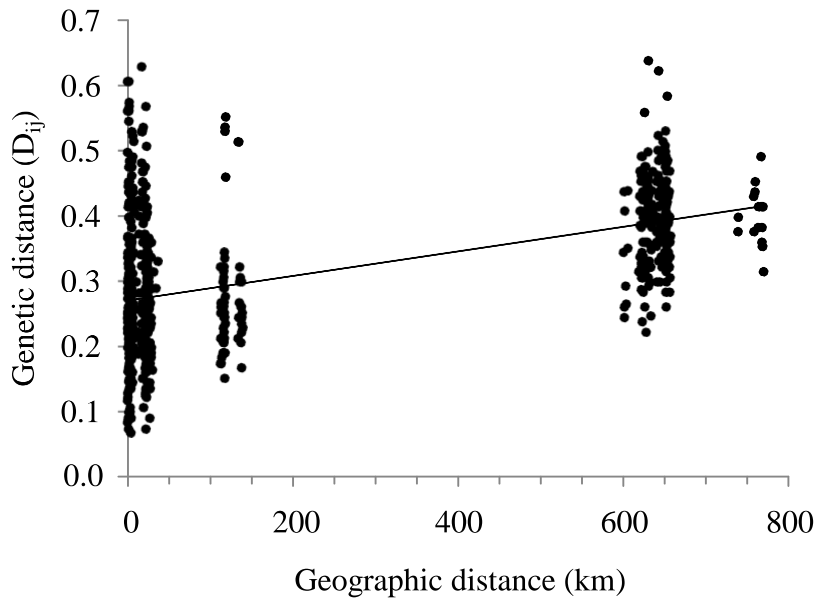
Table 8. Pairwise F_{st} values between snubfin dolphin sampling regions calculated with microsatellite (below diagonal) and mtDNA data (above diagonal). Significant values (*P < 0.05. and **P < 0.01) after Bonferroni corrections are marked with an asterisk.

	Hinchinbrook	Townsville	Keppel Bay
Hinchinbrook		-0.274	-0.200
Townsville	0.017		0.071
Keppel Bay	0.228*	0.226**	

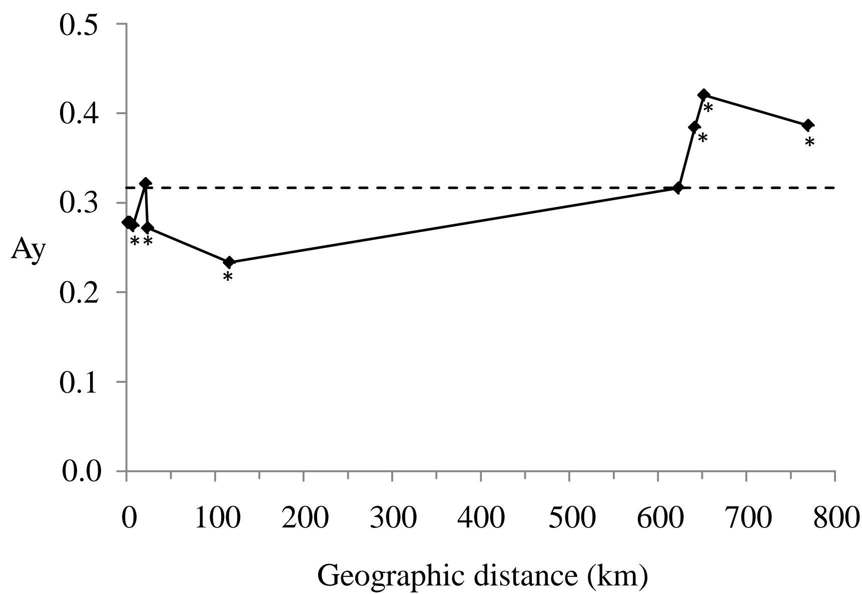
The posterior probabilities [Ln P(D)] results from STRUCTURE and ΔK for data with and without prior information on sampling location indicated that the most likely number of distinct genetic populations ranged from two to three (Fig. 5). The mean membership proportion (*Q*) indicated that at K = 2, most individuals where strongly assigned to one of the two clusters identified (Fig. 6). The membership proportion (*Q*) of Hinchinbrook and Townsville samples to cluster (1) was high, with *Q* = 0.88 for both, while samples from Keppel Bay were assigned with high proportion to cluster 2 (Keppel Bay, *Q* = 0.93).

Sex-biased dispersal

In total 14 females and 20 males were analysed in FSTAT. There was no indication of sex-biased dispersal. FST and FIS values were similar for females and males (FST for females = 0.15, males = 0.23; P = 0.26; FIS for females = 0.04, males = 0.02, P= 0.90). Differences between the mean and the variance of the Assignment Index for females and males were also not significant (mAIc for females = -0.59, males = 0.41, P = 0.44; vAIC for females = 5.26, males = 21.78, P = 0.25).

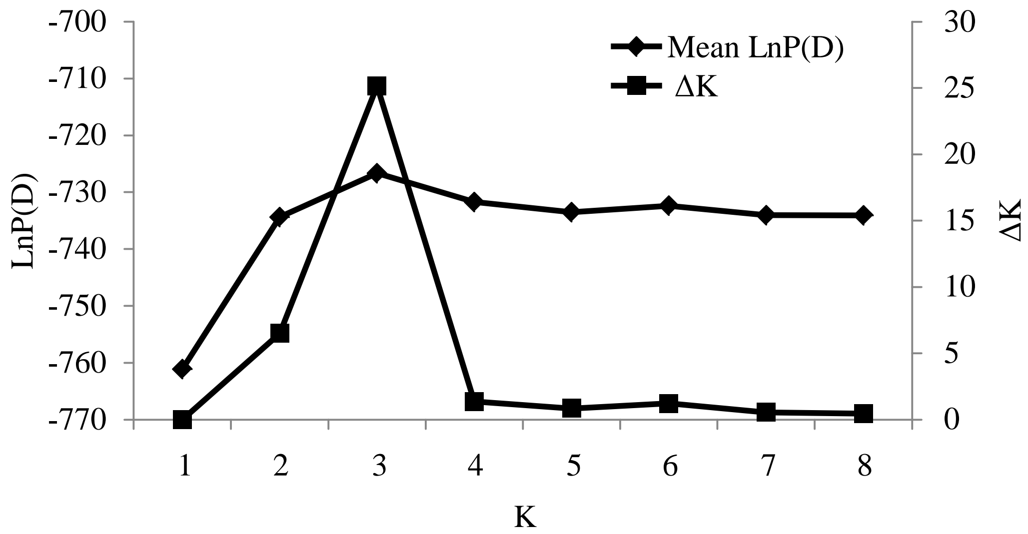


A)

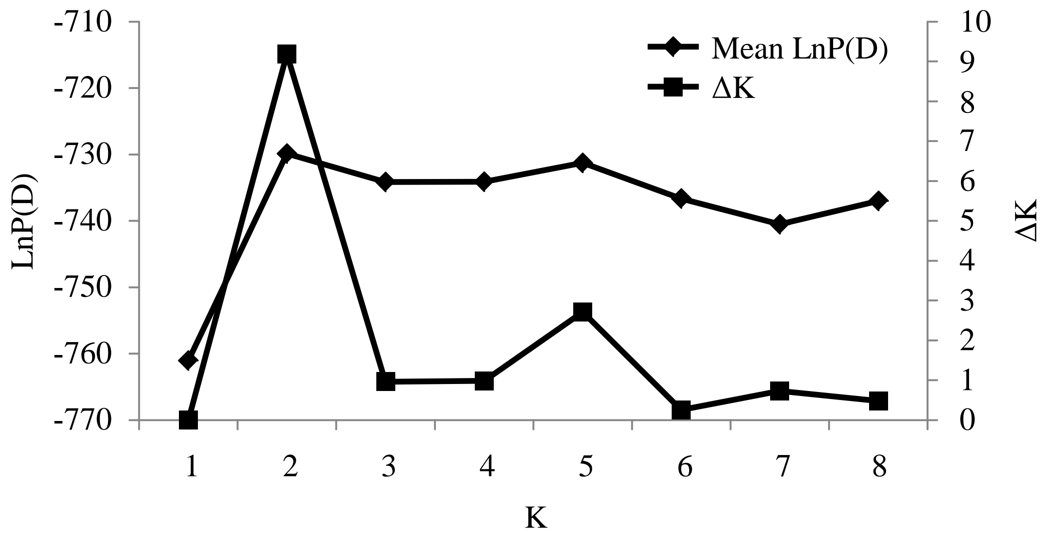


B)

Figure 4. Results of Mantel Test (A) and spatial autocorrelation analyses (B) of snubfin dolphins along the Queensland coastline. Spatial autocorrelation analyses were performed using 10 distinct geographic distance classes. A_y quantifies the average pairwise genetic distances between samples that fall within the boundaries specified for distance class y. Horizontal lines indicate the average value of A_y for the data set. Distance classes in spatial autocorrelations that showed significantly larger or smaller values at the $\alpha = 0.05$ level than average are marked with asterisks.

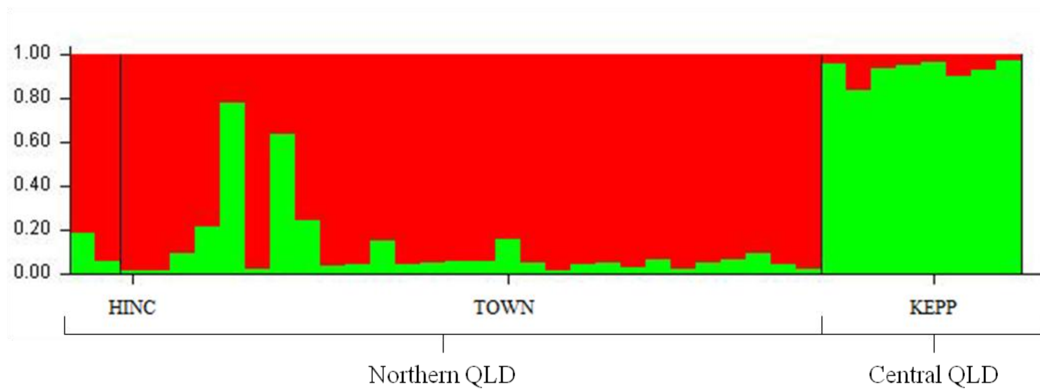


A)



B)

Figure 5. STRUCTURE output of posterior probabilities [Ln P(D)] and ΔK (a measure of the second order rate of change in the likelihood of *K*) of snubfin dolphins data for each value of K (the number of putative populations) under models without (A) and with (B) prior information on sampling location.



A)

B)

Figure 6. Summary plots of genetic clustering analysis in STRUCTURE for snubfin dolphins sampled in three localities (distinguished by black lines) along the east coast of Queensland. Each column represents one individual and colours correspond to the percentage of assignment to each cluster A) K = 2, B) K =3. The most likely number of genetic clusters in the data set was identified as two: Northern Queensland and Central Queensland. Sampling sites within these two clusters are on the X-axis organized from north (left) to south (right): HINC = Hinchinbrook; TOWN = Townsville; and KEPP = Keppel Bay.

Migration rates

Simulations in BAYEASS show that in instances where there is no information in the data, show that in instances where there is no information in the data, the mean and 95% confidence interval for the non-migration rates are 0.833 (0.675-0.992) and 0.0837 (0,-0.261) for the migration rates. Confidence intervals obtained from the data set for Townsville and Keppel Bay were considerably smaller than those obtained randomly (Table 9), suggesting that sufficient information was available to reliably estimate migration rates for these two populations. Due to the small number of samples from Hinchinbrook migration and non-migration rates are unreliable. Both Townsville and Keppel Bay populations had a high proportion of individuals identified as non-migrant (96–97%) with very low migration rates.

Table 9. Mean (95% CI) posterior distributions for migration rates among snubfin dolphins in Queensland calculated with the program BAYESASS. Values along the diagonal (bold) are the proportion of individuals each generation that are not migrants. Simulations show that in instances where there is no information in the data, the mean and 95% confidence interval for the non-migration rates are 0.833 (0.675-0.992) and for the migration rate are 0.0837 (0,-0.261)

Migration rate	To		
From	Hinchinbrook	Townsville	Keppel Bay
Hinchinbrook	**0.750 (0.669-0.909)**	0.189 (0.046-0.310)	0.061 (0.002-0.187)
Townsville	0.017 (0-0.056)	**0.975 (0.932-0.999)**	0.009 (0-0.035)
Keppel Bay	0.018 (0-0.078)	0.018 (0-0.082)	**0.963 (0.876-0.999)**

Discussion

Our results indicate considerable levels of genetic differentiation between most populations of humpback and snubfin dolphins sampled to date along the east coast of Queensland. Low levels of genetic diversity are characteristic within all snubfin and humpback dolphins' localities. Humpback dolphins within the urban coast of Queensland appear to be differentiated into at least three highly distinct populations (Northern Queensland, Central Queensland, and South Queensland). Further subdivision of these populations is evident based on significant pairwise F_{st} values , low levels of migration rates between most humpback dolphin sampling locations and no obvious sex bias in dispersal.

Similarly, population differentiation among Snubfin dolphin localities is clear between populations in the north (Hinchinbrook and Townsville) and south of Queensland (Keppel Bay). There appears to be very low migration rates between these two regions and there is no indication of sex bias in dispersal.

Isolation by geographic distance appears to be partly responsible for the genetic structure observed in both snubfin and humpback dolphins. Population of humpback dolphins separated by 380 km or more appear to be significantly more differentiated than expected from random. It is also clear that at the spatial scale of pour sampling for snubfin dolphins, north and central QLD populations separated by at least 600km are clearly differentiated.

Conclusions and management implications

Defining population boundaries is essential to the formulation of effective conservation plans, especially for highly mobile species such as dolphins. Altogether, our data suggest that humpback and snubfin dolphin populations along the urban coast of Queensland are genetically differentiated into at least two to three distinct genetic clusters: Northern, central and south Queensland. Our results also highlight that further subdivisions within these clusters are evident for humpback dolphins. The low migration rates of dolphins between these major areas suggest that populations of

snubfin and humpback dolphins from these three areas should be considered as separate entities and considered independently for further actions towards their conservation and management. These findings have important conservation and management implications for both species, especially in light of the recent endemic status of both species to Australian waters, the low population estimates found for populations of both species in north and southern Queensland, and increasing human-related threat to these dolphins in the region due to coastal zone development.

Long-term standardized collection and analysis of biopsy samples from under represented areas across the dolphins range in Queensland will be critical to determine the fine spatial population structure patterns of these coastal dolphins across the state waters. This will be particularly useful to guide future research effort on these species and to inform the planning and management of inshore waters in remote and urban regions of Queensland.

References

Amos B, Hoelzel AR, 1991. Long-term preservation of whale skin for DNA analysis. In: Genetic ecology of whales and dolphins (Hoelzel AR, Donovan GP, eds). Cambridge: International Whaling Commission; 99-103.

Beasley I, Robertson KM, Arnold P, 2005. Description of a new dolphin, the Australian Snubfin dolphin *Orcaella heinsohni* sp. n. (Cetacea, Delphinidae). Mar Mamm Sci. 21:365-400.

Berube M, Palsboll P, 1996. Identification of sex in cetaceans by multiplexing with three ZFX and ZFY specific primers. Mol Ecol. 5:283-287.

Bouzat JL, 2000. The importance of control populations for the identification and management of genetic diversity. Genetica. 110:109-115.

Bouzat JL, Cheng HH, Lewin HA, Westemeier RL, Brawn JD, Paige KN, 1998. Genetic evaluation of a demographic bottleneck in the greater prairie chicken. Conserv Biol. 12:836-843.

Chapuis M-P, Estoup A, 2007. Microsatellite Null Alleles and Estimation of Population Differentiation. Mol Biol Evol. 24:621-631.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK, 2000. Considering evolutionary processes in conservation biology. Trends Ecol Evol. 15:290-295.

Drummond AJ, Ashton B, Cheung M, Heled J, Kearse M, Moir R, Stones-Havas S, Thierer T, Wilson A, 2009. Geneious v4.7, Available from **http://www.geneious.com**.

El Mousadik A, Petit RJ, 1996. High level of genetic differentiation of allelic richness among populations of the argan tree (*Argania spinosa* (L.) Skeels) endemic to Morocco. Theoretical & Applied Genetics. 92:832-839.

Evanno G, Regnaut S, Goudet J, 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol. 14:2611-2620.

Excoffier L, Laval G, Schneider S, 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics. 1:47-50.

Falush D, Stephens M, Pritchard JK, 2003. Inference of Population Structure Using Multilocus Genotype Data: Linked Loci and Correlated Allele Frequencies. Genetics. 164:1567-1587.

Forney KA, Gilpin ME, 1989. Spatial structure and population extinction: a study with *Drosophila* flies. Conserv Biol. 3:45-51.

Frankham R, 1995. Inbreeding and extinction: A threshold effect. Conserv Biol. 9:792-799.

Fraser DJ, Bernatchez L, 2001. Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Mol Ecol. 10:2741-2752.

Frère CH, Hale PT, Porter L, Cockcroft VG, Dalebout ML, 2008. Phylogenetic analysis of mtDNA sequences suggests revision of humpback dolphin (*Sousa* spp.) taxonomy is needed. Mar Freshw Res. 59:259-268.

Gilson A, M. S, Levine KF, Banks JD, 1998. Deer gender determination by polymerase chain reaction: validation study and application to tissues, bloodstains, and hair forensic samples from California. Calif Fish Game. 84:159–169.

Goudet J, 2002. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from **http://www.unil.ch/izea/softwares/fstat.html**. .

Goudet J, Perrin N, Waser P, 2002. Tests for sex-biased dispersal using bi-parentally inherited genetic markers. Mol Ecol. 11:1103-1114.

Guo SW, Thompson EA, 1992. Performing the Exact Test of Hardy-Weinberg Proportion for Multiple Alleles. Biometrics. 48:361-372.

Hanski I, 1998. Metapopulation dynamics. Nature. 396:41-49.

Hoelzel AR, Dahlheim M, Stern SJ, 1998. Low genetic variation among killer whales (*Orcinus orca*) in the eastern North Pacific and genetic differentiation between foraging specialists. J Hered. 89:121-128.

Jefferson TA, Karczmarski L, 2001. *Sousa chinensis*. Mammalian Species. 655:1-9.

Jefferson TA, Waerebeek K, 2004. Geographic variation in skull morphology of humpback dolphins (*Sousa* spp.). Aquatic Mammals. 30:3-17.

Krützen M, Barré LM, Möller LM, Heithaus MR, Simms C, Sherwin WB, 2002. A biopsy system for small cetaceans: darting success and wound healing in *Tursiops* spp. Mar Mamm Sci. 18:863–878.

Krützen M, Valsecchi E, Connor RC, Sherwin WB, 2001. Characterization of microsatellite loci in *Tursiops aduncus*. Mol Ecol Notes. 1:170-172.

Miller MP, 2005. Alleles In Space (AIS): Computer Software for the Joint Analysis of Interindividual Spatial and Genetic Information. J Hered. 96:722-724.

Moritz C, 1994. Defining 'Evolutionarily Significant Units' for conservation. Trends Ecol Evol. 9:373-375.

Nater AK, Anna M., Krützen M, 2009. New polymorphic tetranucleotide microsatellites improve scoring accuracy in the bottlenose dolphin <i>Tursiops aduncus</i>. Molecular Ecology Resources. 9:531-534.

Nei M, Tajima F, Tateno Y, 1983. Accuracy of estimated phylogenetic trees from molecular data. II. Gene frequency data J Mol Evol. 19.

Palsboll PJ, Berube M, Allendorf FW, 2007. Identification of management units using population genetic data. Trends Ecol Evol. 22:11-16.

Park SDE, 2001. Trypanotolerance in West African cattle and the population genetic effects of selection. Dublin: University of Dublin.

Parra GJ, 2006. Resource partitioning in sympatric delphinids: Space use and habitat preferences of Australian snubfin and Indo-Pacific humpback dolphins. J Anim Ecol. 75:862-874.

Parra GJ, 2007. Observation of an Indo-Pacific humpback dolphin carrying a sponge: object play or tool use? Mammalia. 71:147-149.

Parra GJ, Azuma C, Preen AR, Corkeron PJ, Marsh H, 2002. Distribution of Irrawaddy dolphins, *Orcaella brevirostris*, in Australian waters. Raffles Bull Zool. Supplement 10:141-154.
Parra GJ, Corkeron PJ, 2001. Feasibility of using photo-identification techniques to study the Irrawaddy dolphin, *Orcaella brevirostris* (Owen in Gray 1866). Aquat Mamm. 27:45-49.
Parra GJ, Corkeron PJ, Marsh H, 2004. The Indo-Pacific humpback dolphin, *Sousa chinensis* (Osbeck, 1765), in Australian waters: a summary of current knowledge. Aquat Mamm. 30:197-206.
Parra GJ, Corkeron PJ, Marsh H, 2006a. Population sizes, site fidelity and residence patterns of Australian snubfin and Indo-Pacific humpback dolphins: Implications for conservation. Biol Conserv. 129:167-180.
Parra GJ, Schick RS, Corkeron PJ, 2006b. Spatial distribution and environmental correlates of Australian snubfin and Indo-Pacific humpback dolphins. Ecography. 29:396-406.
Pichler FB, Dalebout ML, Baker CS, 2001. Nondestructive DNA extraction from sperm whale teeth and scrimshaw. Mol Ecol Notes. 1:106-109.
Pritchard JK, Stephens M, Donnelly P, 2000. Inference of population structure using multilocus genotype data. Genetics. 155:945-959.
Raymond M, Rousset F, 1995. An exact test for population differentiation. Evolution. 49:1280-1283.
Shinohara M, Domingo-Roura X, Takenaka O, 1997. Microsatellites in the bottlenose dolphin *Tursiops truncatus*. Mol Ecol. 6:695-696.
Slatkin M, 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution. 47:264-279.
Thompson JD, Higgins DG, Gibson TJ, 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl Acids Res. 22:4673-4680.
Tilman D, May RM, Lehman CL, Nowak MA, 1994. Habitat destruction and the extinction debt. Nature. 371:65-66.
Van Ooseterhaut C, Hutchinson WF, Wills DPM, Shipley P, 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes. 4:535 - 538.
Wilson GA, Rannala B, 2003. Bayesian Inference of Recent Migration Rates Using Multilocus Genotypes. Genetics. 163:1177-1191.
Wood CC, Gross MR, 2008. Elemental conservation units: Communicating extinction risk without dictating targets for protection. Conserv Biol. 22:36-47.

Attachment 2.

Final report on biopsy sampling activities of snubfin and humpback dolphins in Western Australia 2010.

Murdoch University

Lars Bejder and Simon Allen

The information summarized in this progress report is a component of a larger collaborative research project funded by the Australian Marine Mammal Centre entitled: *Population genetics and phylogeography of Australian snubfin and humpback dolphins: defining appropriate management units for conservation.*

Here, we report on Murdoch University's responsibilities as outlined in the original AMMC agreement. Simon Allen has joined Murdoch University's efforts on this project subsequent to the execution of the final AMMC agreement. Thus, outcomes reported here reflect a joint contribution by Bejder and Allen.

Below, we report on specified responsibilities as outlined in the Memorandum of Understanding (MOU) between collaborators of the overall project.

Specifically, we report on permits, biopsy effort and preliminary photo-identification data and budget.

1. Apply for any permits required for surveying and biopsying coastal dolphins in Exmouth Gulf and Ningaloo Marine Park (Western Australia).

Three permits were obtained in order to carry out biopsy sampling for Sousa and Orcaella in Western Australia. These permits were:

a. Murdoch University Animal Ethics Approval (Permit # NS2295/09) b. Department of Conservation and Land Management permit: **REGULATION 4**: CONSERVATION AND LAND MANAGEMENT REGULATIONS 2002, AUTHORITY TO ENTER CALM LAND AND/OR WATERS. Permit No. CE002566.

c. Department of Conservation and Land Management permit: **REGULATION 17**: WILDLIFE CONSERVATION ACT 1950, LICENCE TO TAKE FAUNA FOR SCIENTIFIC PURPOSES. Permit No. SF007046.

2. Organise and coordinate biopsy sampling in Exmouth Gulf and Ningaloo Marine Park (Western Australia).

Biopsy sampling of Sousa was successfully carried out in Exmouth Gulf (n=24 samples) and Coral Bay (n=3 samples). Furthermore, sampling was successfully carried out at three additional locations: Dampier (n=20), Port Hedland (n=1) and Broome (n=15). See Table 1 and Figures 1-4. All samples have been successfully delivered to Guido Parra, Celine Frere and Michael Krutzen for genetic analyses.

Table 1. Locations of all biopsy samples of Sousa and Orcaella (Coral Bay, Exmouth, Dampier, Port Hedland and Broome).

General Location	Species	Lat	Long	No. of samples
Exmouth	Sousa	-21.84043	114.17922	1
Exmouth	Sousa	-21.97981	113.9244	1
Exmouth	Sousa	-21.84812	114.01267	1
Exmouth	Sousa	-22.0126	114.12922	1
Exmouth	Sousa	-21.85242	114.00827	2
Exmouth	Sousa	-21.86028	114.00679	1
Exmouth	Sousa	-21.80558	114.0841	1
Exmouth	Sousa	-21.93551	113.93198	1
Exmouth	Sousa	-21.87687	114.16	1
Exmouth	Sousa	-21.8619	114.17112	1
Exmouth	Sousa	-21.85908	114.17316	1
Exmouth	Sousa	-21.79764	114.18845	1
Exmouth	Sousa	-21.79383	114.18855	1
Exmouth	Sousa	-21.81585	114.06831	1
Exmouth	Sousa	21.828	114.02998	1
Exmouth	Sousa	-21.83014	114.03978	4
Exmouth	Sousa	-21.833014	114.03978	1
Exmouth	Sousa	21.97486	113.922152	1
Exmouth	Sousa	-21.90055	113.93153	1
Exmouth	Sousa	-21.99797	113.91984	1
Coral Bay	Sousa	-23.01981	113.79839	1
Coral Bay	Sousa	-23.0769	113.73344	2
Dampier	Sousa	-20.55882	116.78843	1
Dampier	Sousa	-20.68461	116.65076	1
Dampier	Sousa	-20.55857	116.67790	1
Dampier	Sousa	-20.55855	116.67789	1
Dampier	Sousa	-20.55853	116.67788	1
Dampier	Sousa	-20.45842	116.82857	1
Dampier	Sousa	-20.50301	116.81299	2
Dampier	Sousa	-20.52234	116.80238	1
Dampier	Sousa	-20.64943	116.45070	1
Dampier	Sousa	-20.65430	116.62830	1
Dampier	Sousa	-20.52757	116.80917	1
Dampier	Sousa	-20.51467	116.68333	1
Dampier	Sousa	-20.51250	116.80691	3
Dampier	Sousa	-20.62421	116.69537	2
Dampier	Sousa	-20.62425	116.69517	1
Dampier	Sousa	-20.62426	116.69507	1
Port Hedland	Sousa	-20.29846	118.58525	1
Broome	Orcaella	-17.99379	122.28246	2
Broome	Orcaella	-17.98494	122.33023	1
Broome	Orcaella	-17.98094	122.27494	1

Broome	Orcaella	-17.98670	122.26026	1
Broome	Orcaella	-17.98795	122.29423	3
Broome	Orcaella	-17.98795	122.29422	1
Broome	Orcaella	-17.98795	122.29420	1
Broome	Orcaella	-17.98767	122.35188	2
Broome	Orcaella	-17.99223	122.31358	3

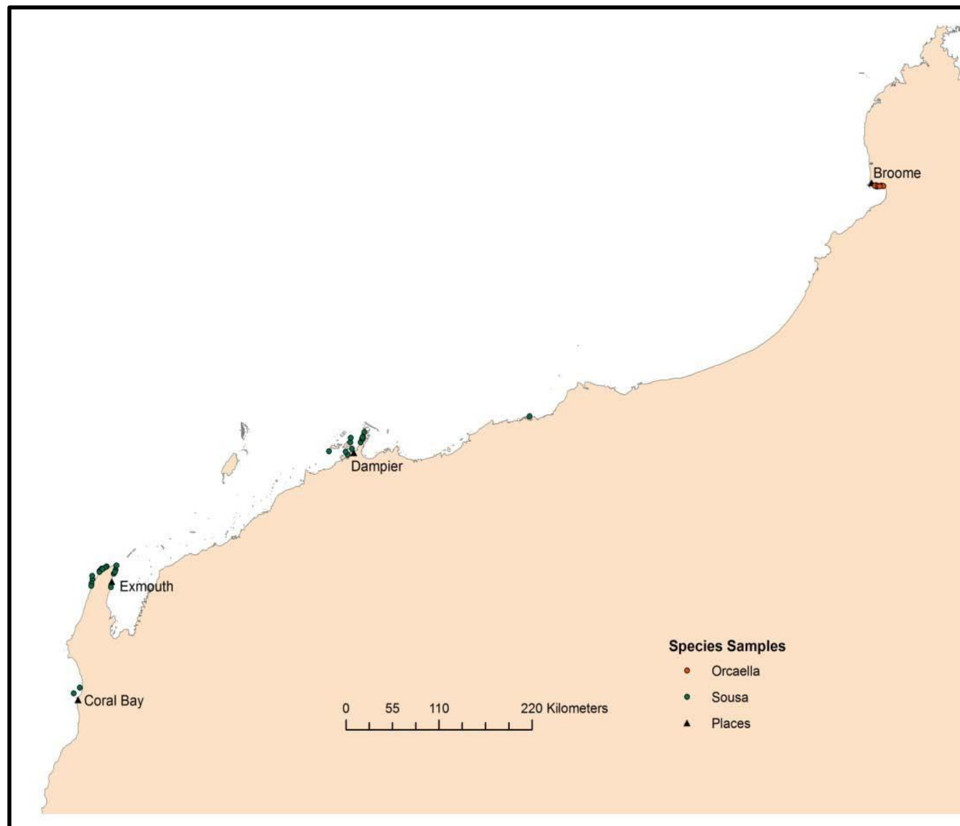


Figure 1. Location of all biopsy samples (Sousa and Orcaella) collected in Coral Bay, Exmouth, Dampier, Port Hedland and Broome.

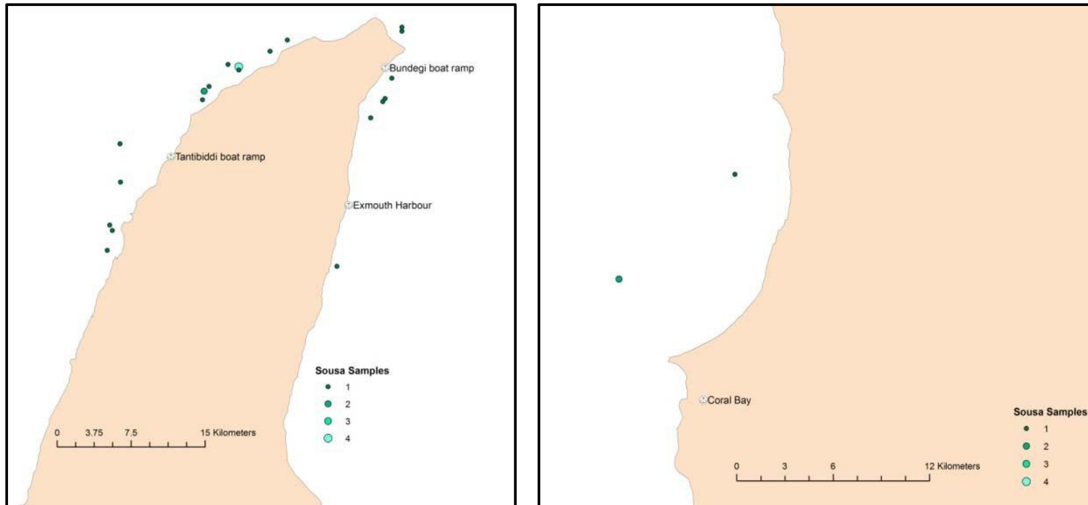


Figure 2. Sousa biopsy samples collected in Exmouth (left) and Coral Bay (right) areas.

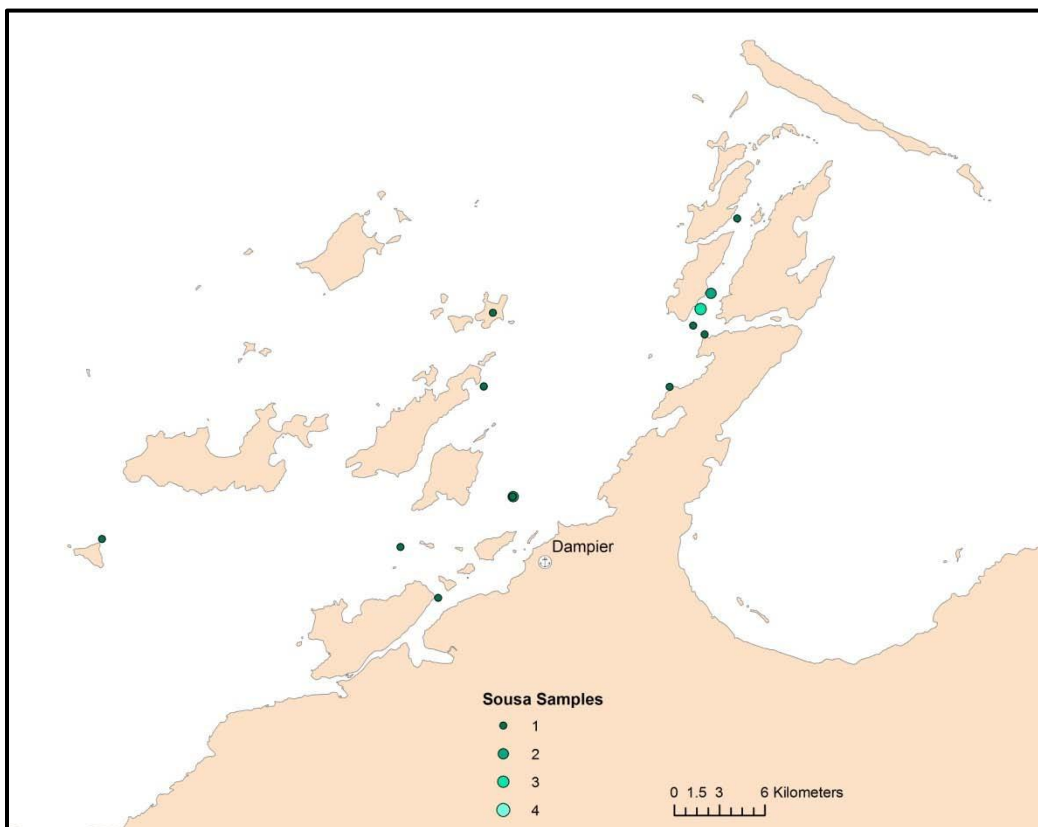


Figure 3. Sousa biopsy samples collected in Dampier

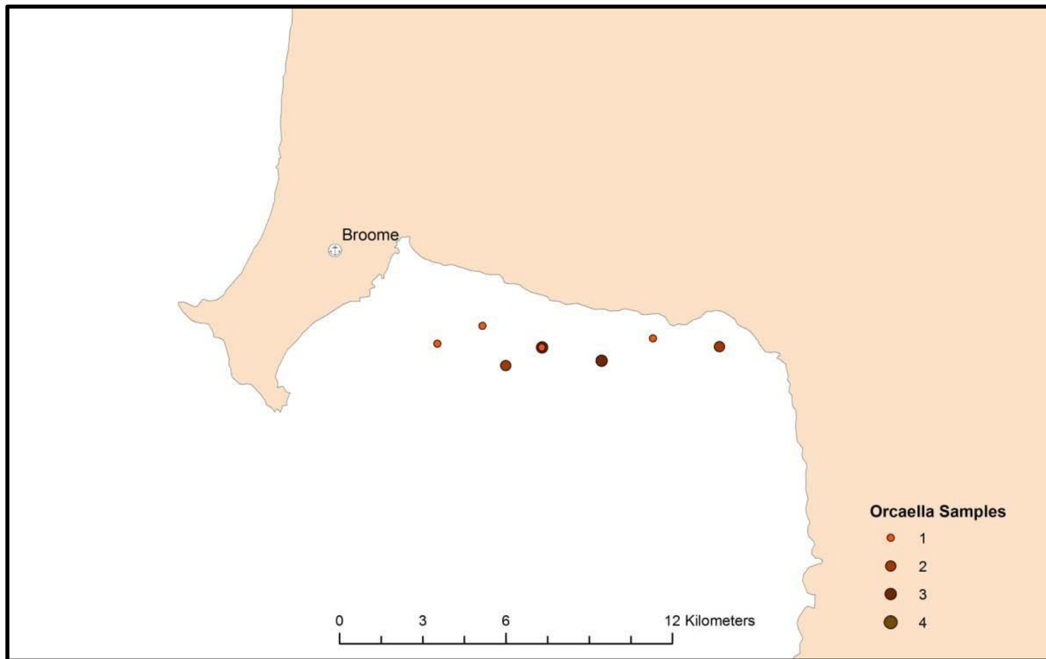


Figure 4. Orcaella biopsy samples collected in Broome.

Photo-identification effort in Coral Bay and Exmouth:

We compiled a preliminary photo-identification catalogue of Sousa from both Coral Bay and Exmouth (Figures 5 and 6). Photo-identification images were obtained during biopsy sampling efforts. The priority of our photo-identification effort was to capture images of biopsied individuals. However, we also endeavored to obtain images of other Sousa present in the focal group. Preliminary results of none-dedicated photo-identification efforts indicate that only a limited proportion of Sousa were identified in these two areas.

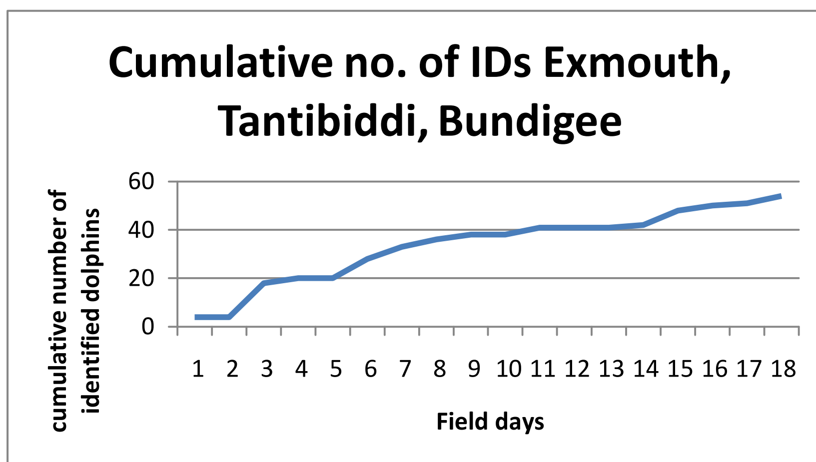


Figure 5. Cumulative discovery curve of identified Sousa in Exmouth during 18 days of biopsy field effort. Our research vessel was launched from three different locations in the Exmouth area (Exmouth harbor, Tantibiddi boat ramp and Bundigee boat ramps).

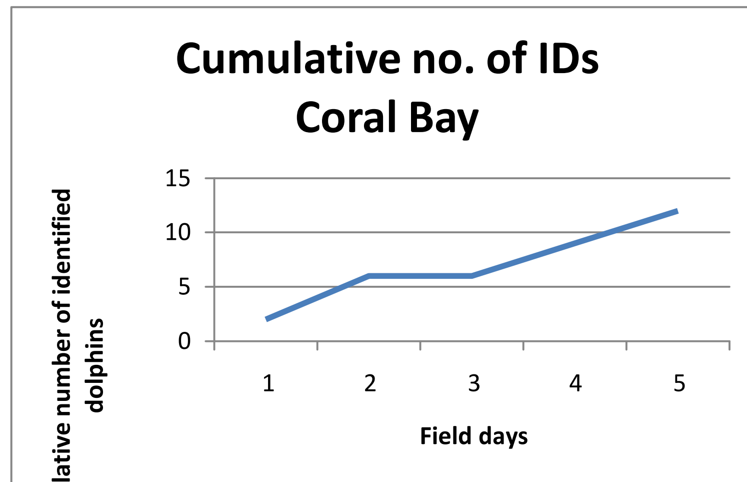


Figure 6. Cumulative discovery curve of identified Sousa in Coral during 5 days of biopsy field effort.

Attachment 3.

Final report on biopsy sampling activities of snubfin and humpback dolphins in Western Australia 2010-kimberley coast biopsy sampling

Deborah Thiele , Marequus Pty Ltd

1. Project progress and objectives achieved

Completed Stage I objectives for sampling in Roebuck Bay. Sampling at northern site to be completed in Stage II, 2010 – 11 as outlined in the MOU between collaborators on this project.

A total of 35 snubfin dolphin samples collected from Roebuck Bay, Broome, WA and sent on to Celine for genetic analysis.

Five samples already existing from 2008 sampling by DT and CP; A

further15 samples collected by CP & DT late July 2010; and Fifteen

samples collected by Simon Allen in early and late July 2010.

2. Milestones and timeframes met

The 2009/10 sampling in the Kimberley was completed by the end of July 2010.

3. Delays affecting project

Poor weather conditions meant that we had to extend planned field trips and make multiple trips to Broome to complete the Stage I objectives.

Our initial field work was scheduled for the week 22 July to 1 August at the end of our survey field work. The field team (DG) stayed on to assist with biopsy. During this week the winds were too strong to safely go out on the bay. CP went to Cape Leveque 2/8 and agreed to return 7th August and stay on for a week of biopsy if weather improved. Accommodation in Broome was extended to 14th August for that purpose ($650 week). The weather did not improve and CP returned to Darwin 8/8 after weather reports made it clear that the weather conditions would be too poor during 7th August to 14th August. In an attempt to avoid the need for another field trip that would involve additional travel expenditure we again extended accommodation in Broome into the first week of September and delayed our own departure from Broome (DT & DG). CP agreed on leaving that she would return if weather conditions improved, but they did not.

DT returned to Broome in February (at no cost to AMMC grant) for meetings and organised for a biopsy sampling trip if weather suitable (CP to come from Darwin if weather conditions were suitable). We had one day of good conditions between 20 – 30 knot wind systems and so DT did not get CP to come to Broome as we would not have been able to get out on the water in the predicted conditions.

In July 2010 we organised another biopsy field trip and the weather conditions were excellent. We obtained 15 samples of snubfin dolphins over 4 days. The accommodation, travel & 4wd hire costs reflect the need for extensions to our original field trip and multiple trips caused by poor weather.

Attachment 4.

Final report on biopsy sampling activities of snubfin and humpback dolphins in the Northern Territory

Carol Palmer, Research Scientist, Biodiversity Division, Department of Natural Resources, Environment, the Arts and Sport

1. Project progress and objectives achieved

The Northern Territory project component completed a total of 60 days of boat-based surveys during the period April 2010 to October 2010 at 2 study sites (Darwin Harbour (which includes Shoal Bay) and Port Essington situated at Cobourg). In total 10 *Orcaella heinsohni* and 4 *Sousa chinensis* biopsy samples were collected (and 3 tissue samples from stranded *S. chinensis*) (Table 1).

Table 1. Biopsy samples of snubfin (Orcaella) and humpback dolphins (Sousa) collected in the Northern Territory.

Skin samples	***Orcaella***	***Sousa***
Darwin Harbour	3	3
Cobourg	7	0
Strandings (Darwin Harbour & Nhulunbuy)	0	3
Total	**10**	**6**

Darting *O. heinsohni* and *S. chinensis* in NT waters proved to be challenging, time consuming and required a different approach to methods adopted for *Tursiops* spp. and other bow-riding dolphins. *Tursiops* spp. and other bow-riding dolphins move in predictable patterns, are usually found in clear water (making it easier to take aim before the animal breaks the surface), animals approach vessels and can be interactive and curious (Kürtzen *et al*. 2002, Bilgmann *et al.* 2006). Darting for bow riding species takes place when animals are travelling parallel to the vessel at slow to moderate speed and at a distance of 4 – 10 m and darting isn’t attempted when animals are socialising or foraging, as movement patterns are unpredictable (Kürtzen *et al*. 2002).

In contrast, *O. heinsohni* and *S. chinensis* don't bow ride, surfacing patterns can be unpredictable, both species can have a low surface profile and they are generally wary of vessels (Dhandapani 1992, Parra *et al.* 2002, 2004). They live in shallow, brackish, turbid estuarine, coastal waters and a number of major river systems (Parra *et al.* 2002, 2004; Palmer *et al.* 2009).

In the Northern Territory, biopsy sampling was undertaken when *O. heinsohni* and /or *S. chinensis* were socialising/foraging as it was during this type of behaviour we could approach within 4 to 10 m of the school. On all occasions the animals movements were unpredictable, water clarity was poor and cohesively animals were in tight groups (<2 m apart). Dolphins were only darted in good sea conditions (Beaufort ≤ 1) and when the vessel was stationary or at a very slow speed < 2km per hour.

We recorded the following responses of the dolphins to darting using modified methods outlined in Kürtzen *et al*. (2002):

- i) Startle / tail slap / dive - returned to surface continued pre-biopsy behaviour
- ii) Tail slap / dive did not return to school moved away from general area of the boat
- iii) Single leap or porpoise
- iv) Multiple leaps and porpoises

Of the 10 *O. heinsohni* biopsied, 6 showed i) No visible response, dolphin continued pre-biopsy behaviour and; four displayed ii) Tail slap / dive did not return to school moved away from general area of the boat.

Sousa chinensis on all three occasions displayed ii) Tail slap / dive did not return to school moved away from general area of the boat.

Anecdotal observations on the longer-term (weeks and months) behavioural impacts of biopsying on both species during foraging / socialising behaviours, suggests ongoing boat avoidance behaviour at the two study sites in the NT. This is in contrast to Krützen *et al*. (2002) where no impacts on biopsied *Tursiops spp*. when travelling was recorded, and the recent findings of Kiszka *et al.* (2010). However, Kiszka *et al.* (2010) suggests spinner dolphins (*Stenella longirostris*) had stronger reactions to biopsy sampling when resting or socialising and it would be preferable for biopsy sampling not to be undertaken during those behaviours. However, no long-term boat avoidance behaviours were observed.

2. Milestones and timeframes met

The Northern Territory component of the project met all stated milestones and timeframes

3. Delays affecting project

There were no delays as such, though weather conditions and avoidance behaviour by both