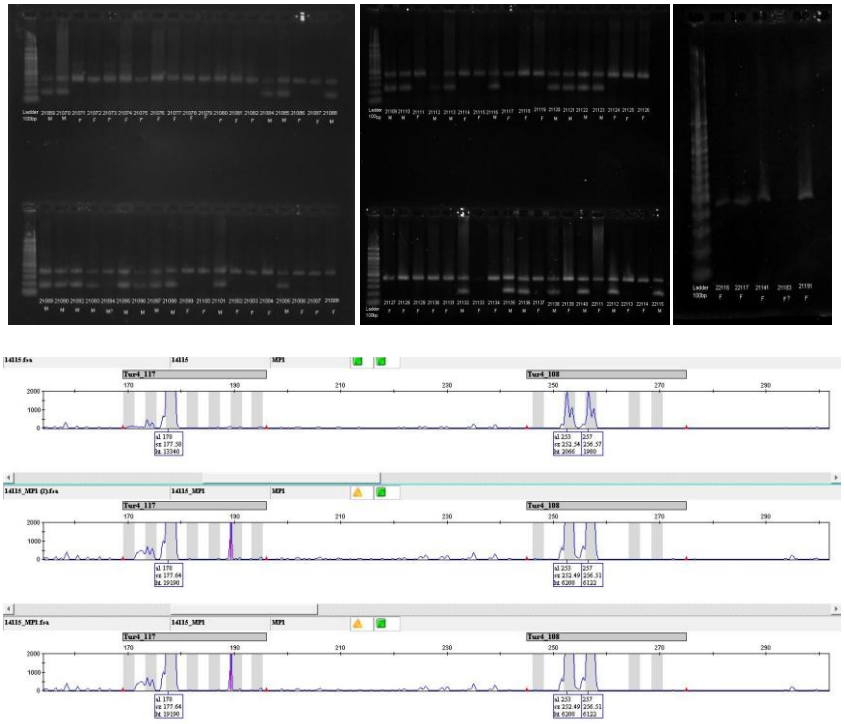
Third Project Report for Consultancy Agreement

CA14000085

Increase understanding of the status of the Australian snubfin and Australian humpback dolphins within Port Curtis and Port Alma



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This report has been produced for the Ecosystem Research and Monitoring Program Advisory Panel as part of Gladstone Ports Corporation's Ecosystem Research and Monitoring Program. The study is being undertaken under a Consultancy Agreement (CA14000085) between Gladstone Ports Corporation and Southern Cross University to monitor and increase understanding of the status of the Australian snubfin and humpback dolphins within Port Curtis and Port Alma.

This publication has been compiled by the Marine Ecology Research Centre, Southern Cross University.

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Abbreviations and acronyms

HCB	Hexachlorobenzene
PCBs	Polychlorinated biphenyls
PBDEs	Polybrominated diphenyl ethers
PAHs	Polycyclic aromatic hydrocarbons
DDTs	dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic acid
mtDNA	Mitochondrial Deoxyribonucleic acid
PCR	polymerase chain reaction
dNTPs	nucleoside triphosphate
^{15}N	nitrogen-15
^{13}C	Carbon-13
ZFX & SRY	Zinc finger X-chromosomal protein & sex-determining region Y

1 Executive Summary

- A total of 64 biopsy samples, 34 snubfin dolphins and 30 Australian humpback dolphins, have been collected during the 2014 and 2015 survey seasons.
- DNA was successfully extracted from 59 of the 64 collected samples.
- The following genetic analyses have been conducted for all 59 samples: a) sex determination, b) amplification of a fragment of the mtDNA control region, and c) genotyping of 27 microsatellite loci.
- Three samples of each species are being used to build RADSeq libraries following a protocol adapted in this study.
- To investigate the diet and potential dietary partitioning and niche segregation between species and populations, we analysed 51 samples for stable isotopes, 23 humpback and 28 snubfin dolphins.
- The project sampling target for Australian snubfin dolphins has been reached. Whereas to meet analysis requirements for the Australian humpback dolphins we aim to collect a total of 13 biopsy samples during the remaining 2016 survey season.

2 Scope of Work

2.1 Overall project objectives for CA14000085

The purpose of this ERMP project, as stated in the ERMP scope of work, is to increase the understanding of the status of the Australian humpback dolphin, *Sousa sahulensis*[1] (Jefferson & Rosenbaum 2014), and the Australian snubfin dolphin, *Orcaella heinsohni* (Beasley et al. 2005) in the Port Curtis and Port Alma regions by considering and extending on previous baseline programs over the period 2014-2016.

More specifically the contractor is required to conduct the following studies in the Port Curtis and Port Alma regions, including the Narrows:

Objective 1: Biannual mark-recapture (photo-identification) surveys of *Sousa sahulensis* and *Orcaella heinsohni* over the period 2014-2016 using protocols that are aligned with the best practice protocols developed by the national coastal dolphin network.

Objective 2: Population genetics using mitochondrial and nuclear markers building on the work conducted to date by: (a) biopsy sampling and analysis of specimens from free-ranging *Sousa sahulensis* and *Orcaella heinsohni*, and (b) analysis of tissues collected opportunistically from the carcasses of these species from this region.

Objective 3: Toxicology analyses of trace and heavy metals, metalloids and persistent organic pollutants by: (a) biopsy sampling and analysis of specimens from free-ranging *Sousa sahulensis* and *Orcaella heinsohni,* and (b) analysis of tissues collected opportunistically from the carcasses of these species from this region.

Objective 4: Stable isotope analyses to gain insights into the diets of these species by: (a) biopsy sampling and analysis of specimens from free-ranging *Sousa sahulensis* and *Orcaella heinsohni*, and (b) analysis of tissues collected opportunistically from the carcasses of these species from this region.

B. Use best practice analyses to interpret these data to inform the ongoing assessment and management of the impacts on these species in the Port Curtis and Port Alma regions.

[1] Following the recent morphological and molecular revision of the genus *Sousa,* humpback dolphins found in the waters of the Sahul Shelf from northern Australia to southern New Guinea that were previously included as Indo-Pacific humpback dolphins (*Sousa chinensis*) have now been determined to be a distinct species, renamed the Australian humpback dolphin (*Sousa sahulensis*).

2.2 Objectives of the third report for CA14000085

As part of the contract agreement, in the third report the contractor is requested to present the first and second population estimates and if available a preliminary report on genetic, toxicology and stable isotope analyses. Preliminary population estimates for the first and second sampling seasons were presented in the second project report (Cagnazzi 2015). In the present third report we provide an update on the status of the genetic, toxicology and stable isotope analysis.

3 Methods

3.1 Biopsy sampling protocol

The survey area encompasses approximately 1,147 km^2 of open water, shallow inshore waters, and intricate estuarine systems between Peak Island in the north and Turkey Beach in the south (Fig. 1).

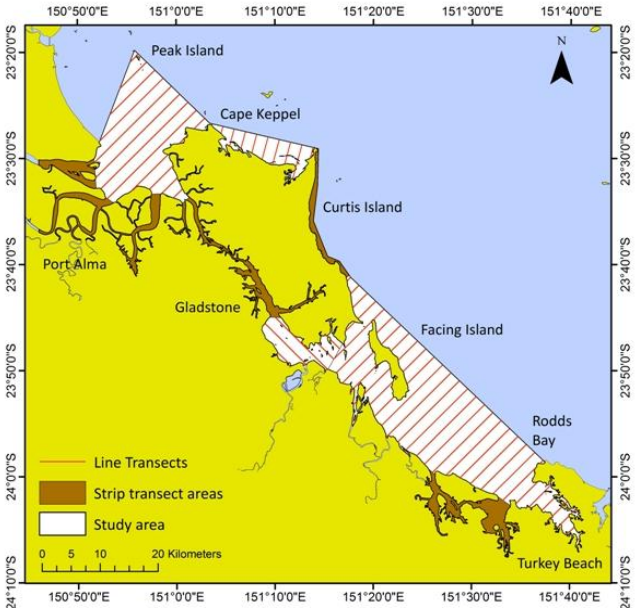

Figure 1. Map of the ERMP study area showing the transects followed during the surveys. In brown, are the areas sampled using strip transect surveys; line transects are shown as red lines.

Biopsy sampling procedures are extremely time consuming, may affect dolphins' behaviour, and bias data collection if conducted during photo-identification mark recapture surveys. Therefore biopsy samples were collected only during boat based surveys dedicated for this purpose. The aim of these biopsy surveys was to search various areas known to be frequently used by dolphins to maximise the opportunity of sighting a group of dolphins and to increase the time spent on biopsy sampling, while decreasing the time spent searching for dolphins.

Biopsy samples (Fig. 2) from Australian humpback and snubfin dolphins (hereafter referred to as humpback and snubfin dolphins) were collected using the PAXARMS biopsy system, which consists of a modified 0.22 calibre rifle with a detachable barrel and a valve to adjust firing pressure in the chamber and biopsy darts. The PAXARMS biopsy system is the safest, most cost-effective, and commonly used method of obtaining skin samples from free-ranging dolphins and was co-developed by the co-investigator Krützen (Krützen et al. 2002). Sampling is undertaken with minimal risk and disturbance, because tissue samples are collected remotely through the use of darts, and animals do not require capturing and/or handling (Krützen et al. 2002). While in the field, all samples were stored in liquid nitrogen, and then transferred to a -80°C freezer once at the University.

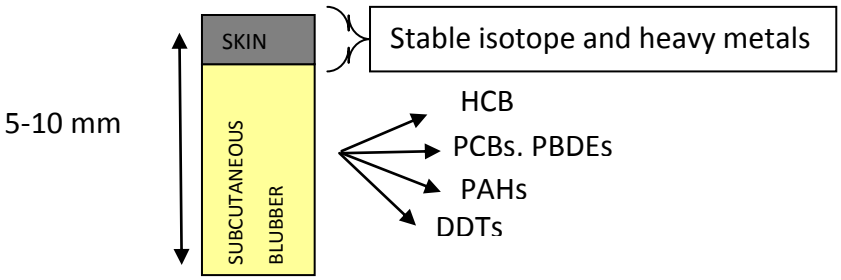

Figure 2. Representation of a biopsy sample showing different sections used in the analyses

After a group of dolphins was sighted, we approached the group to a distance of about 100 m, in order to maintain visual contact without potentially disturbing the dolphins. Dolphins were then approached at a very slow speed, avoiding variation in propeller speed, to a distance of about 50 m. Sighting and photo-identification data were then collected. Once within sampling distance (less than 35 m), darting was attempted only if no boats or people were in visual proximity, there were no dolphin calves in the group to be sampled, and the dolphins showed a predictable behaviour. Biopsies at each sampling site were obtained from individuals from multiple dolphin groups, including solitary individuals. No sample was collected from dependent calves.

3.2 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 (Qiagen, California, USA). 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

& 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.

3.3 Microsatellite genotyping

Biopsy samples were genotyped at 30 microsatellite loci: 10 dinucleotide markers: F10, EV37 (Valsecchi & Amos 1996), KWM12 (Hoelzel et al. 1998), MK3, MK5, MK6, MK8, MK9 (Krützen et al. 2001); and 20 tetranucleotide markers: D8, D22, F10, E12, TUR4_66, TUR4_80, TUR4_87, TUR4_91, TUR4_98, TUR4_105, TUR4_117, TUR4_128, TUR4_138, TUR4_141, TUR4_142, TUR4_153, TUR4_111, TUR4_108, TUR4_132 and TUR4_162 (Nater et al. 2009). 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 GeneMapper software version 4.0 (Applied Biosystems).

3.4 Mitochondrial DNA (mtDNA) screening and sequencing.

A fragment of 428 bp of the mtDNA 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.

3.5 Stable isotopes analysis

Biopsy samples were processed at the Southern Cross University (SCU) Biochemical Laboratory following standard protocols (Tucker et al. 2008, Liu et al. 2015). Between 1-9 mg of epidermal layer was cut from each sample using a stainless steel scalpel which was sterilized between cuts to prevent cross contamination of the samples. These pieces were then transferred into 1.5 ml eppendorf capsules and dried in an oven at 60°C for 24 hours to remove all moisture. The dried samples were ground into a fine powder using a mortar and pestle (which were sterilized with acetone between samples). All samples were lipid-extracted using chloroform methanol solution. Lipid-extracted samples were centrifuged for five minutes at 1000 rpm, the remaining solution was discarded and the powered sample was then placed again in an oven at 60 °C for 24 hours. Between 0.05 and 0.9 mg of powdered sample (depending on the amount of sample left after processing) were wrapped in tin capsules which were analysed using an IRMS against secondary standards of powdered N2 (N), Urea (N) and Glucose (C) to determine the isotopic content of the skin and blubber samples. Isotopic ratios were expressed and reported as per mil (‰) using delta notation (δ):

$$\delta X(‰) = \left(\left(\frac{R_{sample}}{R_{standard}} \right) - 1 \right) \times 1000$$

where X is ^{15}N or ^{13}C, and R_{sample} is the corresponding ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$ in the sample, while $R_{standard}$ is the ratio of stable isotopes in the standard reference material (Jackson et al. 2012).

4 Status of analyses

4.1 Biopsy samples collected and DNA extraction

A total of 34 biopsy samples of snubfin dolphins and 30 of humpback dolphins (15 from Port Curtis, 10 from Port Alma and 5 from Rodds Bay) were collected (Fig. 3, Table A.1a,b) during 42 boat based surveys. Skin samples were collected from free-ranging dolphins only, as no stranded animal was reported within the study area. All available samples were coded and registered (Table A.1a,b) following the protocols applied for the Queensland and Australian wide project on population structure and gene flow in snubfin and humpback dolphins (https://data.marinemammals.gov.au/grants).

DNA was successfully isolated from 59 skin samples, for these samples DNA concentration measured using Nanodrop varied from 3.07 to 653.04 ng/µl (Table A.2). The ratio of absorbance at 260/280 nm and 260/230 nm were approximately 1.8 and 2.2 respectively, which indicates good nucleic acid purity (Table A.2). The DNA concentration in five samples (four humpback dolphins and one snubfin dolphin) was less than 1 ng/µl (Table A.2), both ratios of absorbance were well below expected value for pure DNA, gel electrophoresis also failed to detect any DNA. These samples were composed of about 1 mg of superficial blubber and all available biological material was used in the extraction. Therefore these samples were excluded from the subsequent analysis. For all remaining 59 samples the sex was determined and about 450 bps of the mtDNA control region was amplified. All samples were also genotyped for the complete set of 27 microsatellite loci (Table 1). Additionally three samples from each species were used to construct the RADs (Restriction site associated DNA) libraries to which will follow the generation of thousands of single nucleotide polymorphisms (SNPs) using restriction-site associated DNA (RAD) sequencing. All produced genetic data remain to be checked, tested for quality, scored and analysed.
The nitrogen-isotope values and carbon-isotope values ($\delta^{13}C$ and $\delta^{15}N$) were measured for 47 samples (20 humpback dolphins and 27 snubfin dolphins). For four samples (three humpbacks and one snubfin) only the nitrogen-isotope value was obtained and three samples remain to be analysed while 10 samples didn't meet the analysis requirement (≥ 1 mg of epidermal layer) (Table 1).
A total of 37 samples, 17 snubfin dolphins and 20 humpback dolphins (seven from Port Alma and 13 from Port Curtis) met the requirements (~0. 5 g of epidermal and blubber layers) for toxicology analyses (Table 1).

Table 1. Summary of data collection and status of the analyses against the project schedule. The targets reached are highlighted in bold and underlined.

Project Status	**Genetic analysis**	**Stable Isotopes**	**Toxicology analysis**	**Project schedule**	**Expected completion**
All samples target/analysed	70/59	40/51	40/37	09/2016	09/2016
Snubfin dolphin target / analysed	25/**33**	20/**27**	~15/**17**	09/2016	Completed
Humpback dolphin Port Alma: target / analysed	20-25/9	10/8	~15/7	09/2016	08/2016
Humpback dolphin Port Curtis target / analysed	20-25/17	10/**16**	~15/13	09/2016	07/2016

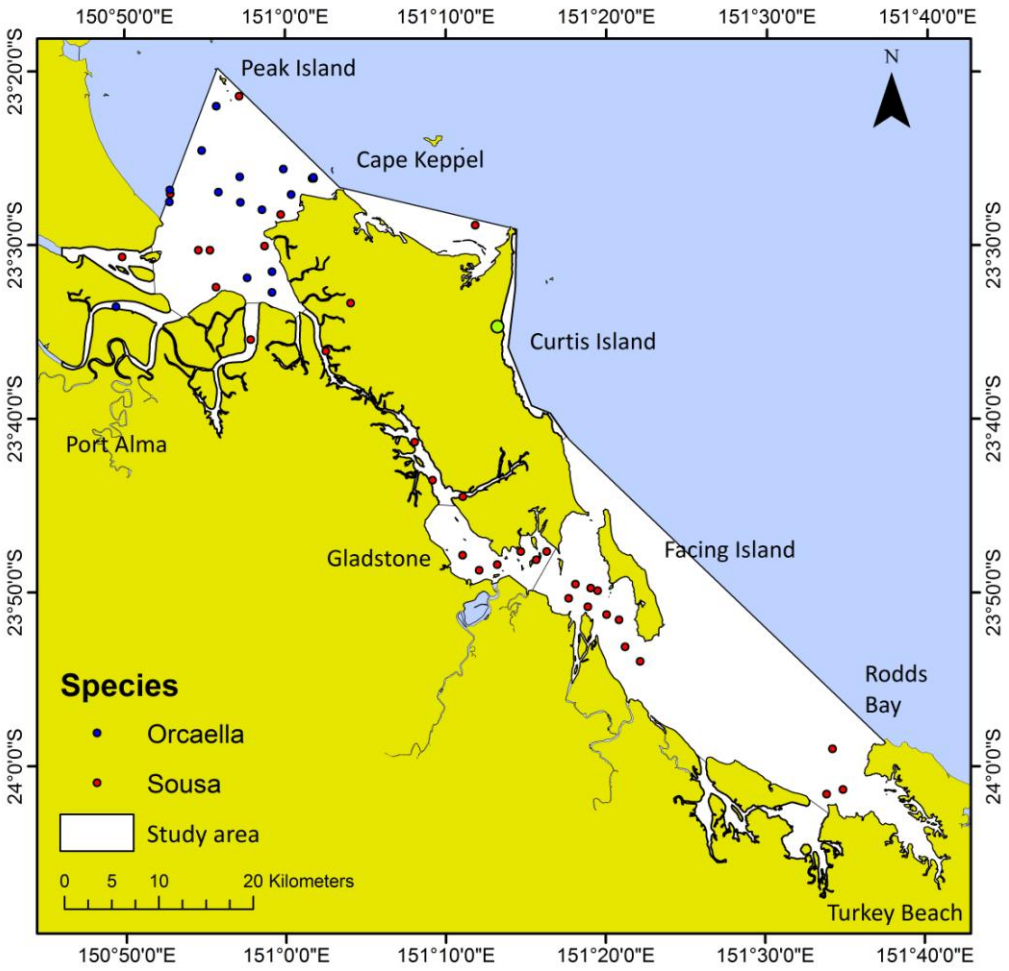

Figure 3. Map of sampling locations of Australian humpback dolphins and snubfin dolphins.

4.2 Discussion on the status of the project.

Overall the project is proceeding well and all the objectives are ahead or within the expected project schedule (Table 1).

All collected samples are listed in Table A.1a,b together with the analyses performed, the difference in the analyses completed across samples is due to the variation in sample weight and composition. While standard genetics analysis can be run on very small particles of skin, stable isotope and toxicology analyses are limited only to samples with a minimum amount of epidermal or blubber layers.

The maximum target of 25 samples established for population genetics was set because variability in allele frequency and expected heterozygosity among replicates decreased with increasing sample size, but these decreases were minimal above sample sizes of 25. Therefore, there appears to be little benefit in sampling more than 25 individuals per population (Hale et al. 2012). While a sample size of more than 10% of the population's effective size (none of the populations studied in

Queensland were larger than 250 individuals) was indicated as target to obtain realistic demographic parameters estimates (Hare et al. 2011).

As it is realistically impossible to collect 75 full size biopsy samples within the project timeframe a lower sampling target of 20 samples per species, was set for toxicology (Objective 3) and stable isotopes analyses (Objective 4). However the number of samples analysed for stable isotopes has already exceeded expectations and by the end of the 2016 survey season a larger sample size is expected to be available for toxicology analyses.

During the first two sampling seasons a total of 34 samples of snubfin dolphins were collected, the minimum number of samples set for each analysis has been exceeded (Table 1), therefore, no biopsy samples of snubfin dolphin will be collected in 2016.

Compared to snubfin dolphins, humpback dolphins tend to have a more unpredictable surfacing pattern and remain at distances of about 30-40 m from the research boat. Despite the challenges, a total of 10 skin samples (genetic analysis = 9, stable Isotopes = 8, toxicology = 7) of this species were collected from Port Alma and 20 from Port Curtis (genetic analysis = 17, stable Isotopes = 16, toxicology = 13).

To meet project objectives during the 2016 survey season, sampling efforts will be focussed towards the collection of a minimum of 10 samples (including eight full size) of humpback dolphins from Port Alma and three (including one full size) from Port Curtis. As a minimum of 25 samples have been collected during a previous sampling season and the subsequent2016 sampling will be conducted on only one species, primarily in one area (Port Alma), it is expected that biopsy sampling will be completed within the scheduled timeframe (September 2016).

In summary, the data required to meet Objective 4 have been produced and any additional samples collected in 2016 will be used to increase sample size. For all available samples, the analyses described under Objectives 2 have been completed (DNA extraction, sex determination, amplification of the mtDNA and the genotype of 27 microsatellite loci) or are currently underway (SNPs) in accordance to the project timeframe. The toxicological tests described in Objective 4 will start only once the sample collection is completed at the end of the 2016 field sampling period. Final results and discussion will be included in the final report.

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Table A. 1a List of all samples collected during the study. All samples are identified with a unique code, the first two numbers (22 & 21) identify the species the following three numbers the individual. In the table: species (OH = Orcaella heinsohni; SS = Sousa sahulensis), sampling location (PA = Port Alma, PC = Port Curtis, RB = Rodds Bay), size of the sample (FULL = epidermal and blubber layers) and whether the analysis was conducted successfully (✓) or not (X).

Code Id	Species	Location	Full sample	DNA extraction	Genetic Analysis	Stable Isotope	Tox
22263	OH	PA	Yes	✓	✓	✓	✓
21259	SS	PA	No	X	X	X	X
21260	SS	PA	Yes	✓	✓	✓	✓
21261	SS	PC	No	X	X	X	X
21274	SS	PA	No	✓	✓	✓	X
21262	SS	PC	Yes	✓	✓	✓	✓
21263	SS	PA	Yes	✓	✓	✓	✓
22264	OH	PA	No	✓	✓	X	X
22265	OH	PA	No	✓	✓	✓	✓
22266	OH	PA	Yes	✓	✓	✓	X
22267	OH	PA	Yes	✓	✓	✓	X
22268	OH	PA	Yes	✓	✓	✓	✓
22269	OH	PA	Yes	✓	✓	✓	X
22270	OH	PA	Yes	✓	✓	✓	✓
22271	OH	PA	Yes	✓	✓	✓	X
21264	SS	RP	Yes	✓	✓	✓	✓
21265	SS	RP	Yes	✓	✓	✓	✓
21266	SS	PC	Yes	✓	✓	✓	✓
21267	SS	PC	Yes	✓	✓	✓	✓
21268	SS	PC	Yes	✓	✓	✓	✓
21269	SS	PA	Yes	✓	✓	✓	✓
22272	OH	PA	Yes	✓	✓	✓	✓
21270	SS	PC	Yes	✓	✓	✓	✓
21271	SS	PC	Yes	✓	✓	✓	✓
21272	SS	RP	NO	✓	✓	✓	X
22281	OH	PA	No	✓	✓	✓	X
22273	OH	PA	No	✓	✓	X	X
22274	OH	PA	Yes	✓	✓	✓	✓
22275	OH	PA	Yes	✓	✓	✓	✓
22276	OH	PA	Yes	✓	✓	✓	✓
22277	OH	PA	No	✓	✓	X	X
22278	OH	PA	Yes	✓	✓	✓	✓
22279	OH	PA	No	✓	✓	X	X
22280	OH	PA	Yes	✓	✓	✓	✓

Table A. 2b List of all samples collected during the study. All samples are identified with a unique code, the first two numbers (22 & 21) identify the species the following three numbers the individual. In the table: species (OH = Orcaella heinsohni; SS = Sousa sahulhensis), sampling location (PA = Port Alma, PC = Port Curtis, RB = Rodds Bay), size of the sample (FULL = epidermal and blubber layers) and whether the analysis was conducted successfully (✓) or not (X).

Code Id	Species	Location	Full sample	DNA extraction	Genetic Analysis	Stable Isotope	Tox
21273	SS	PA	No	✓	✓	X	X
21274	SS	PA	Yes	✓	✓	✓	✓
21276	SS	PC	Yes	✓	✓	✓	✓
21277	SS	PC	No	✓	✓	✓	X
21278	SS	RP	No	✓	✓	✓	X
21275	SS	PC	Yes	✓	✓	✓	✓
21279	SS	RP	No	✓	✓	X	X
22282	OH	PA	Yes	✓	✓	✓	✓
21280	SS	PA	Yes	✓	✓	✓	✓
21281	SS	PC	No	X	X	X	X
21282	SS	PC	Yes	✓	✓	✓	✓
21283	SS	PC	Yes	✓	✓	✓	✓
21284	SS	PC	Yes	✓	✓	✓	✓
22283	OH	PA	Yes	✓	✓	✓	X
22284	OH	PA	Yes	✓	✓	✓	✓
22285	OH	PA	Yes	✓	✓	✓	✓
22286	OH	PA	Yes	✓	✓	✓	✓
22287	OH	PA	No	✓	✓	X	X
22288	OH	PA	Yes	✓	✓	✓	X
22289	OH	PA	Yes	✓	✓	✓	✓
22290	OH	PA	Yes	✓	✓	✓	X
22291	OH	PA	No	✓	✓	X	X
22292	OH	PA	Yes	✓	✓	✓	X
22293	OH	PA	Yes	✓	✓	✓	X
21285	SS	PA	Yes	✓	✓	✓	✓
21286	SS	PA	Yes	✓	✓	✓	✓
22294	OH	PA	No	X	X	X	X
22295	OH	PA	Yes	✓	✓	✓	✓
22296	OH	PA	Yes	✓	✓	✓	✓
21287	SS	PC	No	X	X	X	X

Table A. 3 List of all the samples collected with identification code, DNA concentration and the ratio of absorbance at 260 nm and 280 nm which is used to assess the purity of DNA. A ratio of 260/280~1.8 is generally accepted as "pure" for DNA. Expected 260/230 values are commonly in the range of 2.0-2.2. In red are shown the samples from which it was not possible to extract any DNA.

Sousa **Sample ID**	**DNA conc ng/ul**	**260/280**	**260/230**	***Orcaella*** **Sample ID**	**DNA conc ng/ul**	**260/280**	**260/230**
21259	0.34	0.47	0.07	22263	201.21	1.86	2.53
21260	653.04	1.81	2.21	22264	16.49	1.18	2
21261	0.46	0.42	0.82	22265	28.38	1.6	1.45
21262	298.6	1.75	2.04	22266	63.29	1.83	2.52
21263	235.46	1.81	2.1	22267	129.37	1.88	2.74
21264	401.26	1.8	2.05	22268	492.1	1.71	1.97
21265	320.14	1.82	2.1	22269	63.65	1.75	1.88
21266	541.45	1.74	1.94	22270	140.13	1.57	1.74
21267	115.25	1.95	2.99	22271	340.79	1.71	1.86
21268	226.86	1.72	1.83	22272	389.72	1.79	2.18
21269	239.87	1.9	2.61	22273	120.18	1.5	1.56
21270	316.12	1.78	2.05	22274	323.48	1.63	1.9
21271	181.32	1.85	2.35	22275	23.79	1.76	1.97
21272	81.29	1.9	2.83	22276	268.3	1.75	2.18
21273	336.03	1.83	2.22	22277	26.47	1.62	1.83
21274	288.29	1.74	2.03	22278	113.27	1.65	1.76
21275	107.52	1.75	1.88	22279	43.93	1.72	1.69
21276	512.86	1.63	1.86	22280	195.37	1.72	2.06
21277	19.45	1.73	1.34	22281	107.5	1.68	2.01
21278	12.22	1.44	1.14	22282	178.74	1.72	1.92
21279	175.62	1.77	1.93	22283	18.06	1.88	1.7
21280	99.88	1.95	2.84	22283	102.79	1.82	2.4
21281	-0.14	0.84	-0.04	22284	71.06	1.75	2.05
21282	202.79	1.92	2.83	22285	185.87	1.72	1.82
21283	448.91	1.81	2.38	22286	244.31	1.87	2.72
21284	73.07	1.89	2.72	22287	231.65	1.64	1.72
21285	8.31	1.54	1.53	22288	32.69	1.74	1.84
21286	116.33	1.89	2.83	22289	264.71	1.67	1.85
21287	1.81	0.13	0.11	22290	78.47	1.77	2.41
				22291	19.99	1.77	1.87
				22292	47.51	1.85	2.37
				22293	156.84	1.69	1.74
				22294	0.98	1.12	0.11
				22295	3.07	1.39	1.45
				22296	47.3	1.68	1.59