Southern Cross University

Marine Ecology Research Centre

Fourth 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

Daniele Cagnazzi

Marine Ecology Research Centre

Southern Cross University

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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.

Disclaimer:

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Abbreviations and acronyms

SE	Standard error
SD	Standard deviation
SS	Secondary survey period
95%CI	95% confidence interval
EOM	Extracted Organic Material
OCs	Organochlorines
PCBs	Polychlorinated biphenyl
HCB	Hexachlorobenzene
DDTs	Dichlorodiphenyltrichloroethane
EPA	Environmental Protection Agency
ICP-MS	Inductively coupled plasma-mass spectrometer
Ag	Silver
As	Arsenic
Pb	Lead
Cd	Cadmium
Cr	Chromium
Cu	Copper
Mn	Manganese
Ni	Nickel
Se	Selenium
Zn	Zinc
Hg	Mercury
Fe	Iron
Al	Aluminium
$\delta^{15}N$	Nitrogen isotope ratio ^{15}N:^{14}N
$\delta^{13}C$	Carbon isotope ratios ^{13}C:^{12}C

1 Executive Summary

- In this fourth report, I have summarised data collected from boat-based surveys during 2016 in Port Curtis and Port Alma. All transects were surveyed five times, resulting in five secondary sampling periods. During each of the secondary survey periods at least 577 km of line transects were surveyed to collect data on groups of dolphins encountered.
- In 2016, a total of 96 adult Australian humpback dolphins and 38 adult Australian snubfin dolphins with long-term identifiable marks were photographed. The number of Australian humpback dolphins captured in each secondary survey period always exceeded the minimum capture target (n = 15) to obtain robust and accurate abundance estimates. However, the number of snubfin dolphins was lower than the capture target in three of the five secondary occasions.
- Seven biopsy samples were collected in 2016, reaching a total of 72 samples collected over three years. The overall project target, 70 biopsy samples, has therefore been exceeded. All biopsy samples with the exception of those collected in 2016 have been analysed for stable isotopes, heavy metals and organochlorines. A descriptive summary of the results is presented in this report.

2 Scope of Work

2.1 Overall project objectives for CA130074

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 and Rosenbaum 2014), and the Australian snubfin dolphin, *Orcaella heinsohni* (Marine Mammal ScienceBeasley 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:

[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*).

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.

2.2 Objectives of the second report for CA14000085

As part of the contract agreement, in the fourth report the contractor is requested to present a general summary of the data collected during the 2016 survey season, and a descriptive analysis and summary of the available results from the genetic, toxicology and stable isotope analyses.

In this report we:

1. Summarise sightings and photo-identification data collected during the 2016 sampling season.
2. Present descriptive analysis and summary of stable isotopes analysis
3. Present descriptive analysis and summary of organochlorines and heavy metals analyses

It is not the purpose of this document to provide detailed statistical analyses and discussions on mark recapture analysis, genetic analysis, stable isotope analysis and contaminants levels. This information will be included in the final project report due in June 2017.

3 Methods

3.1 Survey design and sampling protocol

The survey area encompasses about 1,147 km^2 of open water, shallow inshore waters, and intricate estuarine systems between Peak Island to the north and Turkey Beach to the south (Fig. 1). A stratified survey sampling design was developed to increase survey efficiency and ensure a uniform coverage of this highly complex area (Strindberg and Buckland 2004, Thomas et al. 2010). To create the design, the study area was divided into five strata with rather different physical characteristics: Outer Fitzroy River, Inner Fitzroy River, Eastern Curtis Island, Port Curtis and Rodds Bay. Each of these strata was surveyed with a combination of line and strip transects. Line transects were used to survey sufficiently large bodies of waters within the study area, whereas strip transects were applied to survey creeks, rivers, small inlets, and narrow stretches of coastal waters (where line transect surveys are deemed to be inefficient). The total length of the line transects for this design was estimated to be 493 km and strip transect areas were estimated to cover approximately 167 km^2, for a total coverage of 54% of the entire study area.

The sampling design for the Port Curtis and Port Alma region called for a Robust Design Model (Pollock 1982), with a minimum of three primary samples, each consisting of five secondary samples. A primary sample is the combination of five secondary samples completed within a sampling season (May to September of each year). A secondary sample is one complete survey of all navigable line and strip transects. Conditional on weather and operational conditions, it was planned to complete a secondary sample in five surveys with two boats operating contemporaneously. The maximum total length of each secondary sample was expected to vary between seven and 10 days. A comprehensive overview of the sampling design applied in this study was given in the first project report[2] (Cagnazzi 2015a).

[2] The first project report is available online at

http://gpcl.com.au/EnvironmentDocuments/MarineEcologyResearchCenIncreaseUnderstandingoftheStatusoftheAustralianSnubfinandAustralianHumpbackDolphins.pdf

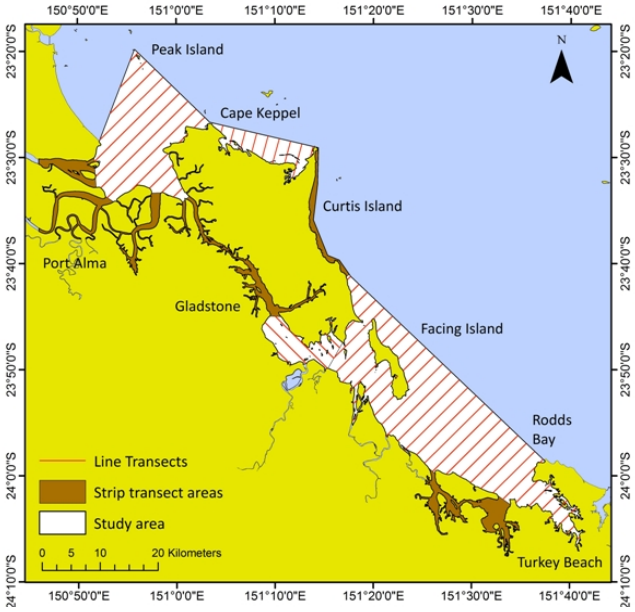

Figure 1. Map of the ERMP study area showing the transects followed during the surveys. In brown are the areas sampled using strip transect surveys; line transects are shown as red lines.

3.2 Data collection

Data was collected following standard procedures applied in boat-based capture-recapture studies for inshore dolphin species (Parra et al. 2006, Cagnazzi et al. 2011). Boat-based surveys were completed at an average speed ranging between 10 and 12 km/hr (about 6 kts). Additional information collected from each survey included date, start and end time, wind direction and speed, sea state, visibility, and total transects surveyed during the day.

During surveys, two observers, one on each side of the boat, searched for dolphin groups while another observer was resting. The three observers were rotated every 30 mins, allowing 30 mins rest for every 60 mins of observation. We defined a dolphin group as any dolphins within 100 m of another and involved in similar behavioural activities. After a group of dolphins was sighted, we initially estimated group size and behaviour from a distance to minimise the influence of the research vessel. Once the first set of data was collected, the group was approached to take photographs of the right and left sides of each dorsal fin. Dorsal fin images were recorded using Nikon and Canon digital cameras equipped with 80-400 mm zoom lenses. Data recorded at each sighting included: species, group size, group composition (adults, juveniles and calves), date, time, geographical location (latitude and longitude), water depth, and behaviour (foraging, travelling, socialising and milling). Detailed definitions are available in Cagnazzi (2015a).

3.3 Analysis of photo-identification data quality and dorsal fin distinctiveness

Nicks, notches and others injuries on the dorsal fin's trailing and leading edges are the most common types of natural marks used in photo-identification studies of dolphins (Urian et al. 2015). These marks are fairly stable over time and allow for the identification of dolphins from pictures taken from either side of the dorsal fin. Other permanent marks, such as mottled patches, may be selected as primary identification marks only if one side of the dorsal fin is used for identification purposes. Scratches and skin disorders are likely to disappear over the course of the study and should not be used.

All photographs collected during the study were graded according to photographic quality and dorsal fin distinctiveness to minimise the introduction of any biases caused by some individuals being more distinguishable than others, and to reduce misidentification (Gowans and Whitehead 2001). All photographs were assigned absolute values based on the following criteria: clarity and focus (2 = in focus, 4 = slightly off focus or 9 = off focus), degree of contrast (1 = dorsal fin clearly distinguishable form the background or 3 = lack of contrast), angle of dorsal fin to the camera (1 = 90°, 2 < 100° or 8 > 100°), dorsal fin visibility and the proportion of the frame filled by the dorsal fin (1 = good & marks visible without zooming in or 5 = poor or marks visible only once zoomed in) (Brooks and Pollock 2011, Tyne et al. 2014). These values were summed to produce an overall image quality score. Images were defined "excellent" with a total score of 5-7, "good" with a score between 8–11 or "poor" with a scores >11 (Cagnazzi 2015a) .

3.4 Sample preparation and stable isotope analysis

Preparation of skin samples followed standard protocols for stable isotope analysis (Browning et al. 2014). Approximately 10 mg of skin was cut from each sample using a stainless steel scalpel which was sterilised with ethanol between cuts to prevent cross contamination of the samples. These skin pieces were then transferred into Eppendorf capsules and dried in an oven at 60°C for 24 hours to remove all moisture. Once dried, samples were ground into a fine powder using a mortar and pestle (which were sterilised with acetone between samples). In order to minimise variance from lipid content (Liu et al. 2015); all samples were lipid-extracted by adding 5 ml of 2:1 chloroform methanol solution to the powdered samples, which were then vortexed for 30 seconds to ensure proper mixing (Post et al. 2007). Lipid-extracted samples were placed into a centrifuge for 5 minutes at 1000 rpm; the remaining solution was discarded and samples were again placed in a drying oven at 60°C for 24 hours to remove residual solvent. Depending on the amount of

sample available after processing, aliquots of 0.05 to 0.9 mg of powdered sample were sealed in tin capsules which were analysed using a Thermo Fisher Delta V plus isotope ratio mass spectrometer. These samples were run against secondary standards of powdered N_2 (Nitrogen), Urea (Nitrogen) and Glucose (Carbon) every 10 cycles to assure quality control during the analysis. Isotopic ratios were transformed into parts per thousand (‰) using delta notation (δ):

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

where δX is ^{13}C or ^{15}N, R sample is the ratio of stable isotopes in the sample and R standard is the ratio of stable isotopes in the standard reference materials (atmospheric nitrogen gas and carbon from Pee Dee Belemnite, a limestone from South Carolina).

The stable isotope ($\delta^{15}N$, $\delta^{13}C$) data for each species were tested for normality (Shapiro-Wilks Test) and homogeneity of variance (non-parametric Levene's test) using the statistical program IBM SPSS statistics 24. Tests revealed normality for all isotopes and for both species except for $\delta^{13}C$ for humpback dolphins which was not normally distributed (P = 0.019). Equality of variance between species (P = 0.483 for carbon and 0.400 for nitrogen) was satisfied; therefore, we used non-parametric tests (Kruskal-Wallis) to investigate interspecific differences in isotope content. Significance level was set at 95% for all statistical tests.

3.5 Analysis of organochlorins levels

Analysis for Hexachlorobenzene (HCB), dichlorodiphenyltrichloroethane (DDTs) and polychlorinated biphenyl (PCBs) was performed according to methods of the U.S. Environmental Protection Agency (EPA 8081/8082 modified by Marsili and Focardi (1997).

In summary, total PCBs (∑PCBs) were quantified as the sum of 30 congeners (IUPAC no. 95, 101, 99, 151, 144, 135, 149, 118, 146, 153, 141, 138, 178, 187, 183, 128, 174, 177, 156, 171, 202, 172, 180, 199, 170, 196, 201, 195, 194, 206), total DDTs (∑DDTs) as the sum of the op' and pp' forms of DDT, DDD and DDE. Contaminant levels were reported using mean, median, standard deviation (SD), standard error (SE) and range (minimum and maximum values).

3.6 Analysis of heavy metals

Analyses of metals were completed at the Environmental Analysis Laboratory at Southern Cross University. Analytes measured in the sample digests included mercury (Hg), arsenic (As), Cadmium (Cd), copper (Cu), iron (Fe), selenium (Se), zinc (Zn), lead (Pb), silver (Ag), chromium (Cr),

manganese (Mn), nickel (Ni), aluminium (Al). Concentrations in the sample digests were measured using an inductively coupled plasma-mass spectrometer (ICP-MS; Perkin Elmer NexION 300D). The instrument was calibrated for each element using a three-point calibration curve, prepared from certified stock solutions, to provide an R2 coefficient of 0.9999 or greater. Calibration standards were analysed at regular intervals to ensure the instrument maintained acceptable linearity and sensitivity criteria (Allen et al. 2012, EAL 2013).

4 Results

4.1 Summary of data collection for primary sample three: May to September 2016

4.1.1 Line-transect surveys

All the five programmed secondary sampling occasions were completed between the 23rd of May and 16th of September, 2016. All of the accessible transects (number of transects = 58) and strip areas were surveyed over a similar number of days of on-water effort and were completed within the maximum of 6 days, a reasonable time to assume the population closed (Table 1) as required by the model design (Cagnazzi 2015a). Other than weather limitations, the difference between the completed and expected area coverage was due to the inaccessibility (water depth below 0.5) of some areas at lower tides. On average, about 8.6 hr of visual survey was completed per day (range: 2-10 hr), with the earliest start at 06:17. and the latest end of the survey at 16:45. All surveys were started in a Beaufort sea state ≤1 and paused or completed with Beaufort sea state ≥3 (or in presence of white caps) to ensure maximum probability of sighting dolphin groups.

Table 1. Study period and effort summary for the 2016 survey season divided by secondary sampling periods (SS).

SS	Dates (2016)	Boat days/ length of SS	Total km covered	Group sightings		
				Humpback	Snubfin	Bottlenose
1	23-05 to 28-05	6/6	713.4	10	17	3
2	7-06 to 10-06	5/5	642.3	11	6	1
3	7-07 to 12-07	6/6	715.8	29	13	0
4	20-07 to 23-07	5/5	577.7	16	4	0
5	10-09 to 15-09	6/6	799.7	22	10	3

4.1.2 Distribution of dolphin sightings and group sizes

During the five secondary periods we surveyed a total of 3,448 km in both on- and off-effort mode, and recorded 87 humpback dolphin groups, 49 snubfin dolphin groups, one mixed-species group of humpback and snubfin dolphins, seven groups of bottlenose dolphins, three single

dugongs and one mother calf pair (Table 1). Recaptures of the same group within one day have not yet been removed from the analysis.

Snubfin dolphins were encountered primarily in the Port Alma section of the study area (Fig. 2), with only one exception being a single snubfin dolphin in a group of seven humpback dolphins sighted in Rodds Bay. Humpback dolphins were sighted throughout the entire study area (Fig. 2). However, compared to the first two survey seasons no humpback dolphins were encountered along the east coast of Curtis Island, while only one group was reported from the east side of Facing Island. For the first time since the study commenced a single humpback dolphin was observed crossing The Narrows.

All the groups of bottlenose dolphins were sighted off Facing Island or near Peak Island, while dugongs were sighted within Port Curtis only (Fig. 3). As a result of the low number of bottlenose dolphins, data analysis was focused only on snubfin and humpback dolphins.

Groups of humpback and snubfin dolphins were encountered in each secondary sample, in water depths ranging from 1 to 23 m, with an average depth of 9.5 m (SE = 0.5 m).

Humpback and snubfin dolphins were found in groups of various sizes with an average of about five dolphins (Mean = 4.9, SE = 0.43, Range = 1-26;) and three dolphins (Mean = 3.4, SE = 0.40, Range = 1-13), respectively.

Travelling and foraging were the most commonly observed behaviours for both species (travelling: humpback = 44; snubfin = 13; feeding/foraging: humpback = 24; snubfin = 27), followed by milling (humpback = 11; snubfin = 1) and socialising (humpback = 7; snubfin = 1). On eight occasions (humpback = 2; snubfin = 6), no behavioural state was assigned.

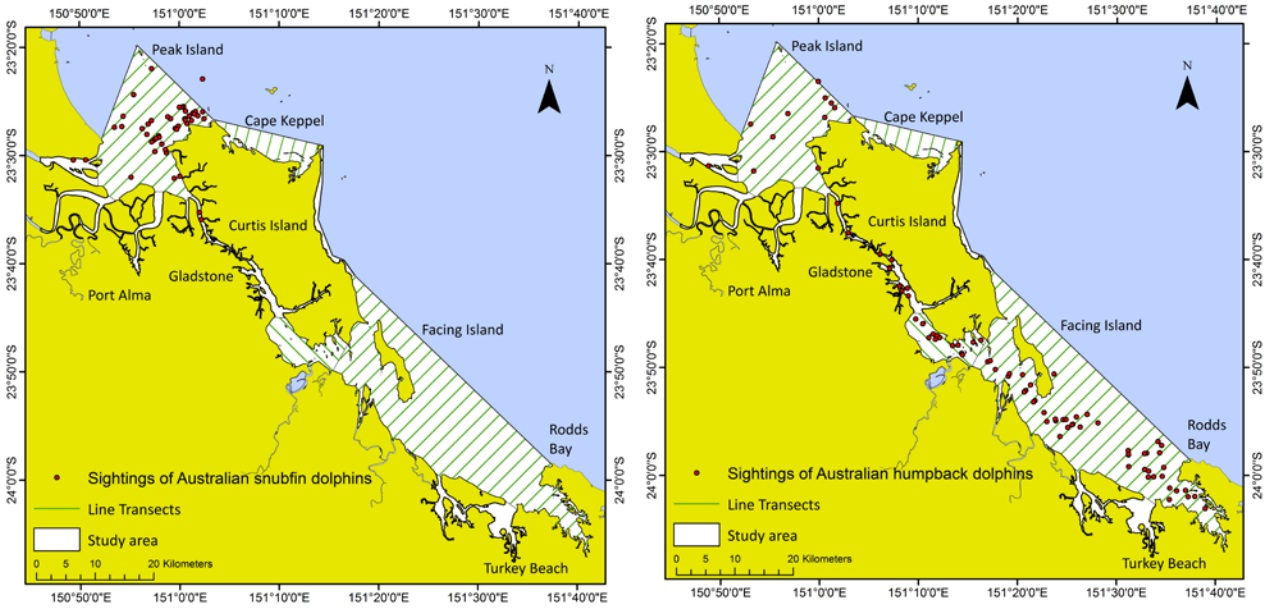

Figure 2 Distribution of groups of Australian snubfin (left) and humpback dolphins (right) sighted in the ERMP survey area during boat-based surveys using line transects in 2016.

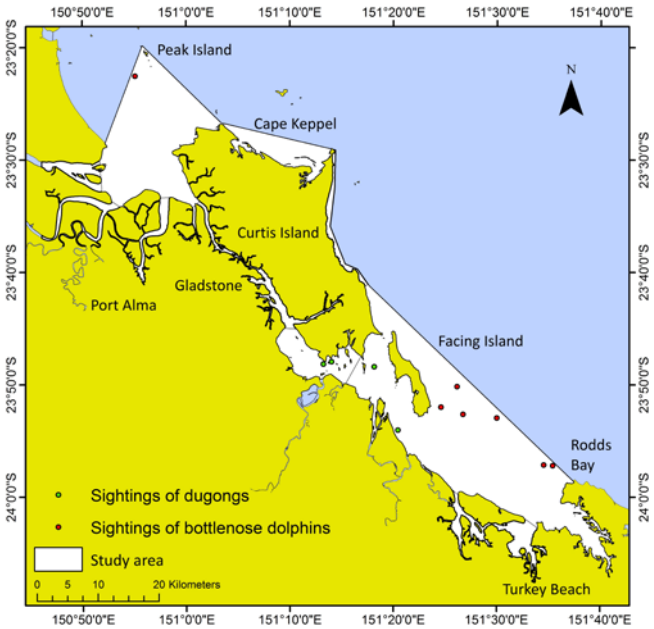

Figure 3 Distribution of groups of other species of marine mammal sighted in the ERMP survey area during boat-based surveys using line transects in 2016.

4.1.3 Summary of photo-identification data

All the images taken during the 2016 season have been analysed and checked for errors. We are currently conducting a revision of all photographs matched in the 2014 and 2015 survey seasons. Overall, in 2016 a total of 14,198 images were taken for both humpback and snubfin dolphins (humpback = 9,149; snubfin = 2,389). Of these, 1,755 were classified as excellent, 2,645 as good and 10007 as poor. From the 4,220 pictures classified as good or excellent, a total of 96 adult marked humpback dolphins and 38 adult marked snubfin dolphins have been identified. In addition to adults, nine juveniles and five humpback dolphin calves and five juvenile snubfin dolphins showed long term identifiable marks. The number of re-sightings of humpback and snubfin dolphins (considering multiple records within secondary occasions) varied from one to seven and one to three respectively. During each secondary capture period (p_n), at least 22 humpback dolphins (p_1 = 29; p_2 = 22; p_3 = 63; p_4 = 41; p_5 = 30) were identified, well above the minimum capture target of 15 individuals required to obtain robust and precise abundance estimates. For snubfin dolphins, the capture target was met only on secondary capture occasions one and five (p_1 = 24; p_2 = 4; p_3 = 7; p_4 = 5; p_5 = 18).

4.2 Descriptive results for stable isotopes analyses

A total of 54 biopsy samples were analysed for stable isotopes (Table 2). Australian snubfin dolphins δ^{13}C (‰) values varied from -18.2 to -13.9 (mean±SD = -15.910±0.845); and δ^{15}N (‰) values varied from 8.9 to 13.3 (mean±SD = 11.160±1.003). Australian humpback dolphins δ^{13}C (‰) values varied from -18.5 to -13.9 (mean±SD = -16.348±1.151); and δ^{15}N (‰) values varied from 9.9 to 13.5 (mean±SD = 11.226±0.879). Snubfin and humpback dolphins showed no apparent significant difference in δ^{15}N values (Kruskal-Wallis, df = 1, X^2 = 0.060412, P = 0.806), but significant differences in δ^{13}C composition were detected (Kruskal-Wallis, df = 1, X^2 = 3.9863, P = 0.046); with snubfin dolphins having a higher mean δ^{13}C than humpback dolphins (Table 2).

The inter-specific similarities in δ^{15}N and differences in δ^{13}C isotopic composition found in this study suggests that snubfin and humpback dolphins feed at similar trophic levels, but differ in the sources of basal resources in their diet. Specifically, snubfin dolphins are more enriched in δ^{13}C, indicative of foraging in more inshore, benthic habitats than humpback dolphins.

Table 2. Mean values (±SD) of δ13C (‰) and δ15N (‰) of Australian snubfin and humpback dolphins.

Species	Sample size (n)	Mean $\delta^{13}C$ (‰) (±SD)	Mean $\delta^{15}N$ (‰) (±SD)
Snubfin	31	-15.910±0.845	11.160±1.003
Humpback	23	-16.348±1.151	11.226±0.879

4.3 Summary results for organochlorine levels

A total of 35 dolphin blubber samples (humpback dolphins = 17; snubfin dolphins = 18) were analysed for an extensive suite of organochlorine compounds (OCs) (Table 3) and 39 skin samples (humpback dolphins = 17; snubfin dolphins = 22) were analysed for heavy metals. Total lipid content averaged 30% wet weight in humpback dolphins and 20% in snubfin dolphins; individual blubber samples were highly variable in lipid content, ranging from 11 to 90% in humpback dolphins and 13 to 31% in snubfin dolphins. Thus, the lipid content can be regarded as the lipid value for all major contaminant groups. ∑PCBs, DDTs and HCB are summarised by species in Table 3. Heavy metals are summarised in Table 4 for humpback dolphins and Table 5 for snubfin dolphins.

Table 3 Descriptive statistics for organochlorines levels (OCs) presented in ng/g of lipid weight from 17 samples of Australian humpback dolphin and 18 samples of Australian snubfin dolphin. In the table EOM% = Extracted Organic Material; H_2O% = proportion of water extracted; SD = standard deviation; SE = standard error; HCB = hexachlorobenzene; DDTs = dichlorodiphenyltrichloroethane; PCBs = polychlorinated biphenyl.

Humpback dolphins	Mean	Median	Minimum	Maximum	SD	SE
H_2O%	51.77	53.76	20.65	60.07	9.19	2.17
EOM %	26.33	21.04	11.18	90.60	19.16	4.29
HCB	32.47	30.70	3.68	89.50	24.31	5.73
DDTs	6965.87	6820.76	1167.91	16351.70	3921.51	924.31
PCBs	5648.94	2960.36	437.94	46838.70	10419.29	2455.85
Snubfin dolphins	**Mean**	**Median**	**Minimum**	**Maximum**	**SD**	**SE.**
H_2O%	56.03	55.43	47.09	62.81	3.79	0.92
EOM %	19.71	16.99	13.75	31.69	5.42	1.31
HCB	12.96	11.57	5.78	40.35	8.53	2.07
DDTs	4152.09	3607.19	1788.59	6964.23	1473.02	357.26
PCBs	3024.03	3150.03	1238.79	5757.36	1262.80	306.27

Table 4 Descriptive statistics for heavy metal levels for 17 samples of Australian humpback dolphins. Results are reported in mg/kg dry weight. In the table SD = standard deviation; SE = standard error;. Ag = silver; As = arsenic; Pb = lead; Cd = cadmium; Cr = chromium; Cu = copper; Mn = manganese; Ni = Nickel; Se = Selenium; Zn = Zinc; Hg = Mercury; Fe = Iron; Al = aluminium.

Metal	Mean	Median	Minimum	Maximum	SD	SE
Ag	~0	~0	~0	~0	~0	~0
As	1.11997	1.064338	0.6	1.880645	0.410889	0.099655
Pb	744.0697	463.6908	8.525203	4037.314	1310.265	317.7859
Cd	2.160784	0.607329	0.056629	13.22975	3.512329	0.851865
Cr	2.414758	3.724787	0.65096	5.534237	1.60161	0.388447
Cu	4.612915	5.081904	2.228151	8.50973	1.943222	0.4713
Mn	1.357426	2.296175	0.254323	3.254162	0.817086	0.198173
Ni	0.86965	2.96969	0.406667	1.914286	0.471061	0.114249
Se	29.41529	23.38292	8.7125	72.1079	16.3242	3.9592
Zn	404.8037	1042.081	245.712	642.7804	108.4209	26.29593
Hg	5.309639	2.655236	1.673864	15.72147	3.875263	0.939889
Fe	68.788	84.6599	24.9026	157.5118	46.05509	11.17
Al	131.01	142.8944	19.9471	337.5136	103.599	25.12644

Table 5 Descriptive statistics for heavy metal levels for 22 samples of Australian snubfin dolphins. Results are reported in mg/kg dry weight. In the table SD = standard deviation; SE = standard error; Ag = silver; As = arsenic; Pb = lead; Cd = cadmium; Cr = chromium; Cu = copper; Mn = manganese; Ni = Nickel; Se = Selenium; Zn = Zinc; Hg = Mercury; Fe = Iron; Al = aluminium.

Metal	Mean	Median	Minimum	Maximum	DS	SE.
Ag	~0	~0	~0	~0	~0	~0
As	1.533816	1.064338	0.546667	3.672727	1.009392	0.215203
Pb	609.9487	463.6908	13.58905	2952.861	789.4915	168.3201
Cd	1.738897	0.607329	0.019415	8.572956	2.410968	0.51402
Cr	4.54019	3.724787	1.072782	15.28661	3.421914	0.729554
Cu	5.28567	5.081904	1.856026	9.05973	2.33575	0.497984
Mn	3.27559	2.296175	0.261014	13.02916	3.389421	0.722627
Ni	3.438162	2.96969	0.601852	9.566667	2.305655	0.491567
Se	26.10539	23.38292	9.431481	59.83824	12.73394	2.714886
Zn	981.0035	1042.081	496.859	1373.652	252.382	53.80801
Hg	2.92075	2.655236	-0.00114	7.216364	1.702007	0.362869
Fe	111.73	84.6599	14.84542	328.3287	80.27502	17.11469
Al	240.5678	142.8944	28.40717	921.1621	237.8971	50.71984

5 Summary of the project status

We were able to survey all transects five times between May to September of each year from 2014 to 2016, resulting in five secondary capture periods and three primary periods. During each secondary occasion we photographed at least 16 adult marked humpback dolphins, exceeding the

minimum capture target (≥ 15 adult marked dolphins) needed to obtain robust and accurate abundance estimates (CV < 0.2). Whereas in the second and third sampling seasons the capture target for snubfin dolphins was achieved only during two secondary sampling occasions. Various methods to minimise the effect of a lower capture rate on the precision of the abundance estimates have been highlighted in the second project report and will be applied in the final report (Cagnazzi 2015b). For example sex has been determined for at least 74% of the adult dolphins in the catalogue, using ten years of genetic and observational data collected by Cagnazzi D. from humpback and snubfin dolphins in Port Curtis and Port Alma (Cagnazzi 2011, Cagnazzi 2013, Cagnazzi et al. 2013). This information can be used to further improve the precision of abundance estimates and to further investigate the population dynamics of both species.

Over the three years of study we conducted 45 biopsy sampling trips, during we collected a total of 72 biopsy samples, exceeding the established target of 70 samples. Only the seven samples (four humpback dolphins and three snubfin dolphins) collected during the 2016 survey season still remain to be analysed. A summary of data collection and status of the analyses against the project schedule is given in Table 6. The majority of the sampling targets established under Objective 2, 3 and 4 have been met, with the only exception being the number of samples of humpback dolphins available for genetic analysis. Nevertheless the genetic dataset developed during this study merged with genetic dataset built during Cagnazzi's PhD study will provide the most extensive genetic database available for these species in Australia. Such alarge dataset will allow the successful estimation of various biological parameters such as effective population size, inbreeding coefficient, gene flow, migration rate, proportion of residence population and sex ratio.

Overall the field work has been successfully completed, whereas only 7 biopsy samples remain to be analysed. Therefore all project objectives will be successfully achieved within the expected project timeframe.

Table 6. Summary of data collection and status of the analyses against the project schedule. The targets reached are highlighted in green, none achieved targets are highlighted in red.

Project Status	**Genetic analysis**	**Stable Isotopes**	**OCs**	**Heavy Metals**	**Project schedule**	**Expected completion**
All samples target/analysed	70/**69**	40/**51**	36/**36**	36/**39**	09/2016	Completed
Snubfin dolphin target / analysed	~30/**36**	~20/**31**	~18/**18**	~18/**22**	09/2016	Completed
Humpback dolphin target / analysed	~40/**33**	~20/**23**	~18/**18**	~18/**17**	09/2016	Completed

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