Increase understanding of the status of the Australian snubfin and Australian humpback dolphins within Port Curtis and Port Alma Final Project Report (CA14000085)

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

Disclaimer:

Table of contents

List of figures

List of tables

Abbreviations, acronyms and symbols

Ag	Silver
AICc	Aikake Information Criterion
Al	Aluminium
ANZECC/ ARMCANZ	Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand
AR	Allele richness
As	Arsenic
bd	Indo-Pacific bottlenose dolphins
c	Probability of recapture after the first capture
Cd	Cadmium
CD	Mean distance to centroid
Cr	Chromium
CR_b	$\delta^{13}C$ range
CRDM	Pollock's Closed Robust Design Model
Cu	Copper
D-0	Non distinctive dorsal fin
D-1	Little distinctive dorsal fin
D-2	Moderately distinctive dorsal fin
D-3	Highly distinctive dorsal fin
DCBP	Decachlorobiphenyl
DDTs	dichlorodiphenyltrichloroethanes
df	Degree of freedom
DHCDMP	Darwin Harbour Coastal Dolphin Monitoring Program
DL	Detection limit
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide triphosphates
DoE	Commonwealth Department of Environment and Energy
ECI	Curtis Island east coastline
EOM%	Extracted organic material
EPA	Environmental Protection Agency
ERMP	Ecosystem Research and Monitoring Program
ERMPAP	Ecosystem Research and Monitoring Program Advisory Panel
F	Female
f(j)	Frequencies
Fe	Iron
F_{IS}	Inbreeding coefficient
Fst	Fixation index
GBRMPA	Great Barrier Reef Marine Park Authority

GOF	Goodness of fit test
GPC	Gladstone Ports Corporation
h, π	Haplotype and nucleotide diversity
HCB	Hexachlorobenzene
hd	Humpback dolphins
Hg	Mercury
H_o, H_e	Observed and expected heterozygosity
HWE	Hardy–Weinberg equilibrium
IAM	Infinite allele model
ICP-MS	Inductively coupled plasma-mass spectrometer
id	Irrawaddy dolphin
ihd	Indo-pacific humpback dolphin
IRMS	Isotope ratio mass spectrometer
IUCN	International Union for Conservation of Nature
IUPAC	The International Union of Pure and Applied Chemistry
K	Genetic population
KS	Keppel Sands
KW	Kruskal-Wallis Test
LnP(D)	Posterior probability of the data
lw	Lipid weight
m	Recent migration rate
M	Male
M(j)	Total caught
M_b	Mark-recapture model with behavioural responses to first capture
M_{bh}	Mark-recapture model with a combination of behavioural responses and heterogeneity in *p*
MCMC	Markov chain Monte Carlo
M_h	Mark-recapture model with heterogeneity in *p*
Mn	Manganese
MNND	Mean nearest neighbour distance
M_o	Mark-recapture model with no variation in *p*
M_t	Mark-recapture model with time varying *p*
M_{tb}	Mark-recapture model with a combination time varying *p* and behavioural responses to first capture
M_{tbh}	Full hierarchical mark-recapture model
mtDNA	Mitochondrial Deoxyribonucleic acid
M_{th}	Mark-recapture model with a combination of time varying *p* and heterogeneity.
MWW	Man-Whitney U Test
n(j)	Animals caught
N_2	Nitrogen
NA	Mean number of alleles

na	Not available
N_e	Contemporary genetic effective population size
NGSS, SGSS	Northern and Southern Great Sandy Strait
nh	Number of haplotypes
Ni	Nickel
NR_b	$\delta^{15}N$ range
OCs	Organochlorines
p	Estimates capture probability
PA	Number of private alleles
PA	Port Alma
PAHs	Polycyclic aromatic hydrocarbons
Pb	Lead
PBR	Potential biological removal
PC	Port Curtis
PCBs	Polychlorinated biphenyls
PCR	Polymerase chain reaction
P_{ID}	Probability of identity
pp	Primary period
PVMs	Population viability models
QAICc	Quasi-Aikake Information Criterion
R	Pearson correlation coefficient
RB	Rodds Bay
RNA	Ribonucleic acid
RSE	Relative standard error
sd	Snubfin dolphins
SD	Standard deviation
SE	Standard error
Se	Selenium
SEA_c	Standard ellipse area
$^{S}H_{UA}$	Shannon's mutual information index
SMM	Stepwise mutation model
sp	Secondary period
TA	Total area
TPM	Two-phased model of mutation
u(j)	newly caught
WBDDP	Western Basin Dredging and Disposal Project
WHT	Whitsundays
ww	Wet weight
ZFX & SRY	Zinc finger X-chromosomal protein & sex-determining region Y
Zn	Zinc

γ”, γ’	Temporary emigration parameters
$\delta^{13}C$	Carbon isotope ratios ^{13}C:^{12}C
$\delta^{15}N$	Nitrogen isotope ratio ^{15}N:^{14}N
^{13}C	Carbon-13
^{15}N	Nitrogen-15
ΔAICc	Differences between two Aikake Information Criterion
δX	Isotopic ratios
Φ_{ST}	Standardized index of genetic differentiation
ϕ	Probability of apparent survival
$\widehat{N}_{\text{total}}$	Total abundance estimate
$\widehat{M}_{\text{marked}}$	Abundance estimates of adult marked humpback dolphins
$\widehat{N}_{\text{lower}}$, $\widehat{N}_{\text{upper}}$	Lower and upper Log-normal confidence intervals for abundance estimates
$\widehat{M}_{\text{P}}$	Estimate proportion of marked individuals
95%CI	95% confidence interval

Executive summary

- The Western Basin Dredging and Disposal Project (WBDDP) was approved by the Commonwealth Department of Environment and Energy (DoEE) in October 2010. The WBDDP was a capital dredging program involving the deepening and widening of existing channels and swing basins and the creation of new channels, swing basins and berth pockets that commenced in May 2011 and was completed in September 2013. The Port Curtis and Port Alma Ecosystem Research and Monitoring Program (ERMP) was developed to acquire a detailed ecological understanding of the marine environment of Port Curtis and Port Alma.
- ERMP approval conditions specify the research that is required on marine megafauna, including dolphins, in the ERMP survey area.
- The ERMP identified four main project objectives that will assist in meeting the ERMP conditions. These objectives were focused on the following aspects: 1. population abundance estimates, 2. genetic population structure, 3. bioaccumulation of contaminants, and 4. feeding habits of Australian humpback and snubfin dolphins in the ERMP survey area.
- The sampling regime applied in this study was based on a Pollock's Closed Robust Design Model with five secondary periods and three primary periods. Boat-based surveys were conducted following a systematic parallel sampling design. During these surveys, the Australian humpback dolphin was the most frequently encountered species, with sightings (n = 249) spread throughout the ERMP survey area. In contrast, Australian snubfin dolphins (n = 122) were only recorded in Port Alma. A large number of sightings of humpback dolphins were observed throughout The Narrows, whereas snubfin dolphins were sighted only in the northern section. The extent to which both species use The Narrows is not yet fully understood.
- Indo-Pacific bottlenose dolphins occurred only sporadically within the ERMP survey area and mostly in open waters.
- This study provides the first population estimates of two coastal dolphin species for the ERMP survey area following the completion of the WBDDP. During the three years study period (2014–2016), we identified a total of 181 and 122 individual Australian humpback and snubfin dolphins, respectively. The population estimates derived herein indicate that about 140–162 adult Australian humpback dolphins and 100–163 adult Australian snubfin dolphins used the ERMP survey area between May and September each year.

- Levels of genetic diversity based on mitochondrial DNA and microsatellite loci were low for both species. Nuclear markers showed strong genetic structure and population differentiation. Bayesian-Markov Chain Monte Carlo approaches grouped Australian humpback dolphins from the ERMP survey area and Australian snubfin dolphins from Port Alma each into single populations, with little to moderate contemporary gene flow (< 25% per generation) between/from nearby populations (Whitsundays and Great Sandy Strait). Significant genetic differentiation was also observed between individual Australian humpback dolphins sampled in Port Alma and Port Curtis. Predicted migration rates for Australian humpback dolphins suggest that Port Alma is the source region for Port Curtis.
- Organochlorine contaminants (ΣPCBs, DDTs and HCB) were extracted from biopsy samples collected from 17 Australian humpback and 18 Australian snubfin dolphins within the ERMP survey area. The results revealed that both species were generally exposed to high levels of these contaminants. In some instances, PCBs and DDTs were present at levels known to affect individual survival and increase the risk of infectious diseases. DDTs, HCB and ΣPCBs values from this study were significantly higher than results from samples collected in the ERMP survey area in 2010 and 2011.
- In this study, 39 epidermis samples (22 Australian snubfin dolphins and 17 Australian humpback dolphins) were analysed for the concentrations of eight essential elements (Zn, Cu, Cr, Se, Ni, Al, Mn, Fe,) and four non-essential elements (Hg, Cd, Ar, Ag). In the majority of samples analysed in this study (63%) some elements exceeded upper baseline values established from biopsy samples collected from free ranging bottlenose dolphins (Bryan et al. 2007, Stavros et al. 2007a). These results indicate a general enrichment of trace element contaminants in Australian humpback and snubfin dolphins from the ERMP survey area from both natural and anthropogenic sources.
- In order to better understand the trophic relationships and resource partitioning among Australian snubfin and humpback dolphins, we compared stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) among these species. Stable isotopes were extracted from skin samples of 31 Australian snubfin and 23 Australian humpback dolphins. Carbon and nitrogen isotope analyses revealed similarity in diets but interspecific differences in habitat use. Tissue carbon concentration was slightly higher on average for Australian snubfin dolphins (mean = -15.910, SD = 0.845‰) than for humpback dolphins (mean = -16.348, SD = 1.151‰), consistent with evidence that Australian snubfin dolphins typically forage in more inshore, benthic habitats than humpback dolphins. Australian humpback dolphins may have a broader trophic niche width compared to Australian snubfin dolphin.

1 Introduction

1.1 Australian humpback and snubfin dolphins: available knowledge

Australian humpback (*Sousa sahulensis*) and snubfin dolphins (*Orcaella heinsohni*) (humpback and snubfin dolphins hereafter) were both recently described as new species, and are endemic to coastal waters of northern Australia and southern New Guinea (Beasley et al. 2005, Mendez et al. 2013, Jefferson and Rosenbaum 2014). A phylogeographic history based on mtDNA proposed that the genus *Sousa* originated in eastern Australian waters, and radiated northwards and westwards into the Indo-Pacific area through the Torres Strait around 8.02 million years ago (Lin et al. 2010). Lin et al. (2010) also proposed that the Australian lineage underwent historical population subdivision or a bottleneck event. Similar phylogeographic information is not available for snubfin dolphins.
Australian snubfin and humpback dolphins are medium sized delphinids. Both are less than 270 cm in length, however snubfin dolphins are substantially lighter with a maximum recorded weight of 133 kg versus 260 kg for humpback dolphins (Arnold and Heinsohn 1996, Beasley et al. 2005, Jefferson and Rosenbaum 2014).
Snubfin dolphins are characterised by a rounded head and blunt rostrum, a small dorsal fin situated on the posterior portion of the body and a subtle three-tone body colouration pattern consisting of a dark brown dorsal cape, a lighter brownish side, and white abdomen (Beasley et al. 2005). Humpback dolphins are mostly grey with a lighter belly, separated by a diagonal cape with indistinct margins. The rostrum, forehead and dorsal fin lose pigmentation and whiten with the age (Jefferson and Rosenbaum 2014). Adult male humpback dolphins exhibit a greater loss of pigmentation on the upper half and leading edge of the dorsal fin than females. In Queensland, many females show light or dark spotting across the entire dorsal fin, which is not so evident in Western Australia (Brown et al. 2016a).
All available evidence suggests that humpback and snubfin dolphins in Australia may exist as metapopulations of small, largely isolated population fragments of less than 200 individuals. No population studied to date is estimated to contain more than 104 mature individuals (Parra and Cagnazzi 2016). Movement patterns among populations are still poorly understood but there is some evidence in support of an isolation by distance model with spatial genetic structure occurring at a scale of more than 300 km. In Western Australia, no movement of either species was observed between a series of sites (Roebuck Bay, Beagle Bay, Cygnet Bay, Cone Bay, Inner Cambridge Gulf) separated by > 200 km over a three years period (Brown et al. 2016b). In the Northern Territory, a relatively high number of humpback dolphins were recaptured across three sites (Boyne

Harbour, Darwin Harbour and Shoal Bay) located along ~ 100 km of coastline in the Darwin region, Northern Territory (Brooks et al. 2017). Recapture of snubfin dolphins across the three sites were substantially lower (number of individuals recaptured across sites = 3) but the high estimates of temporary emigration suggested that considerable movements of individuals occurred in and out each of the three sites towards unobserved sites (Brooks et al. 2017). Strong site fidelity have been reported for humpback and snubfin dolphins along the east coast of Australia (Cagnazzi et al. 2011b, Cagnazzi et al. 2013c), with the majority of individuals regularly returning to the same discrete area from year to year (Parra et al. 2006a).

The Australian humpback and snubfin dolphin are currently listed as "Vulnerable" in the International Union for Conservation of Nature (IUCN) Red List (Parra et al. 2017a, Parra et al. 2017b). In Australia, both species are listed as "cetacean" and "migratory species" under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act). Because of their 'migratory species' listing, both species are also considered "Matters of National Environmental Significance". In Queensland, both species are listed as "Vulnerable" under the *Nature Conservation Act 1992* (Woinarksi et al. 2014). In the Great Barrier Reef Marine Park, humpback and snubfin dolphins are considered priority species for conservation under the Reef 2050 Long-Term Sustainability Plan (Commonwealth of Australia 2015, http://www.environment.gov.au/).

1.2 Purpose of the project

In October 2010, the Commonwealth Department of Environment and Energy (DoEE) approved the Western Basin Dredging and Disposal Project (WBDDP) proposed by the Gladstone Ports Corporation (GPC). The WBDDP consisted of the dredging of Gladstone Harbour with the deepening and widening of existing channels and swing basins, and the creation of new channels, swing basins and berth pockets to provide safe and efficient access to the emerging Liquefied Natural Gas (LNG) industry in the region. The WBDDP also included a 235 ha reclamation site for the development of new shipping berth and offloading facilities and the expansion of the existing Fisherman's Landing wharf. The WBDDP was completed in September 2013 and about 22 million cubic metres of dredging materials were removed in the process (WBDDP 2016).

Among the conditions for approval, the GPC was required to establish The Port Curtis and Port Alma Ecosystem Research and Monitoring Program (ERMP). The ERMP was designed to provide high level information on the health of Port Curtis and Port Alma ecosystems.

The purpose of this project was to "increase understanding of the status of the Australian snubfin and Australian humpback dolphins within Port Curtis and Port Alma by considering and extending on previous baseline programs over the period 2014–2016".

The area covered by the ERMP (hereafter ERMP survey area) extends beyond Port Curtis, Port Alma and the limits of the Gladstone Port inclusive of Rodds Bay, the eastern side of Curtis Island and the mouth of the Fitzroy River (Figure 1).

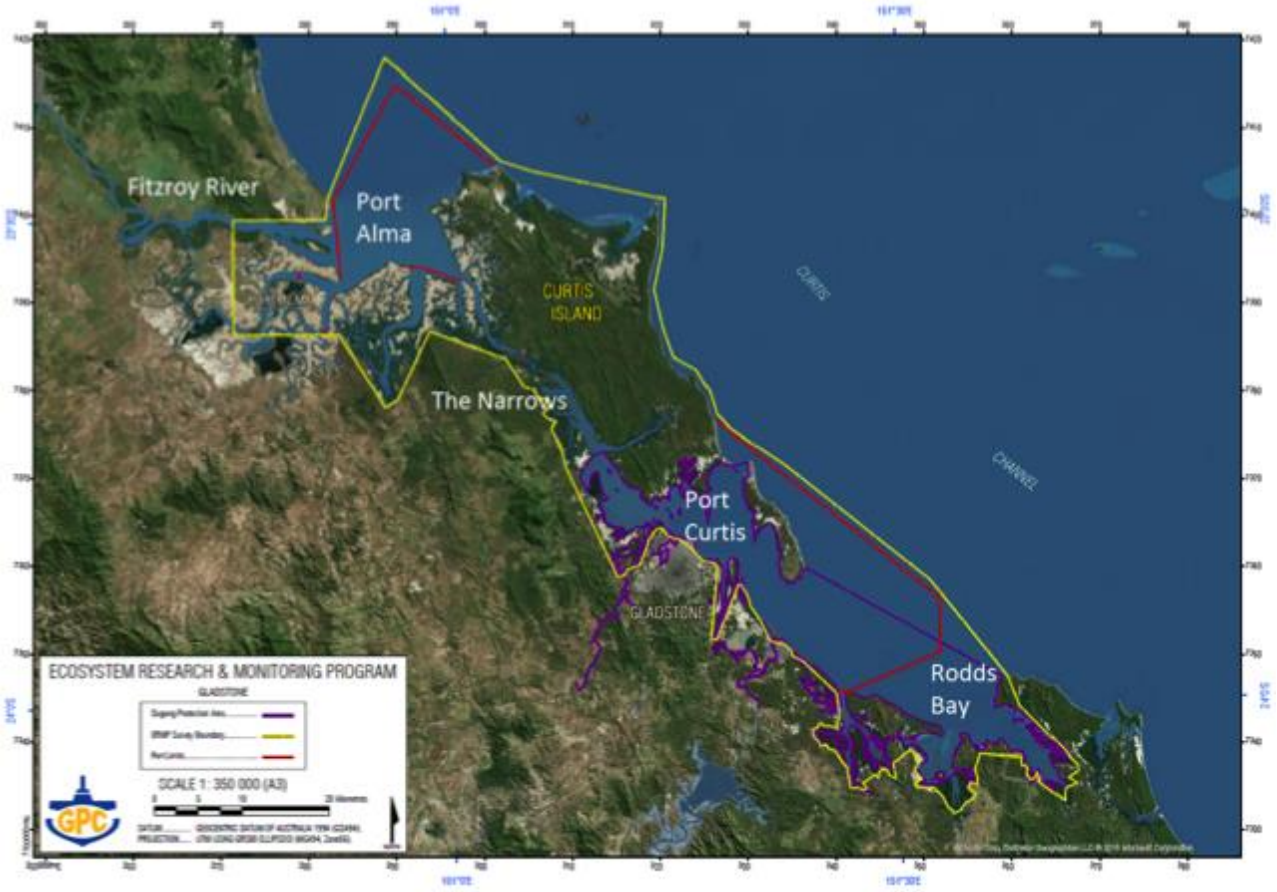

Figure 1. Extent of the ERMP survey area (yellow boundary), Port Limits (Red boundary), and Dugong Protection Area (violet boundary)[1].

[1] Map sourced from the ERMPAP Term of Reference http://www.gpcl.com.au/environment/ermp

1.3 Project objectives

Four main research objectives were identified by the ERMP as priorities to monitor the conservation status and support the management of humpback and snubfin dolphins in the ERMP survey area.

These objectives were:

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 (Section 2).

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 wild *Sousa sahulnesis* and *Orcaella heinsohni*, and (b) analysis of tissues collected opportunistically from the carcasses of these species from this region (Section 3).

Objective 3: Toxicology analyses of trace and heavy metals, metalloids and persistent organic pollutants by: (a) biopsy sampling and analysis of specimens from wild *Sousa sahulensis* and *Orcaella heinsohni,* and (b) analysis of tissues collected opportunistically from the carcasses of these species from this region (Section 4).

Objective 4: Stable isotope analyses to gain insights into the diets of these species by: (a) biopsy sampling and analysis of specimens from wild *Sousa chinensis* and *Orcaella heinsohni*, and (b) analysis of tissues collected opportunistically from the carcasses of these species from this region (Section 5).

Research associated with this study was conducted under permits no. G10/33405.1 and G09/29714.1 issued by the Great Barrier Reef Marine Park Authority (GBRMPA) and permits no. WISP05836609 and WISP05700909 issued by the Department of Environment and Heritage Protection. Ethics approvals were provided by the Southern Cross University (SCU) Animal Care and Ethics Committee (no. 22/11).

1.4 Prior information on Australian humpback and snubfin dolphins in the ERMP survey area.

Previous data on humpback and snubfin dolphins from the ERMP survey area were collected by D. Cagnazzi between 2007 and 2011 as part of a study aiming to assess abundance, distribution, population genetic structure and toxicology of humpback and snubfin dolphins in the Fitzroy River Basin region (Cagnazzi 2011). The Fitzroy River Basin extends from the southern end of the Gladstone Port limits until Keppel Bay and includes Port Curtis, Port Alma, and Curtis Island but excluds Rodds Bay.

Results from those surveys indicated that, based on the distribution of recaptured individuals and their patterns of association, humpback dolphins in Port Curtis and Port Alma formed two geographically discrete social communities named based as Keppel Bay and Port Curtis communities based on their preferred geographic distribution (Cagnazzi 2010). However, preliminary genetic results based on a very small sample size (Port Curtis n = 13; Port Alma n = 9) did not support this separation and suggested the existence of gene flow between the two communities. In both Keppel Bay and Port Curtis humpback dolphins were found year-round with no significant variation in numbers, group size and composition among seasons. Mark-recapture analysis of photo-identification data collected between 2007–2011 suggested that the total number of humpback dolphins using Keppel Bay varied from 115 in 2007 (SE = 7.9, 95%CI = 100-130) to 104 in 2011 (SE = 8.1, 95%CI = 88-120). Total abundance estimates of humpback dolphins in Port Curtis varied from 84 (SE = 5.8, 95%CI = 73-95) in 2007 to 45 (se = 7.7, 95%CI = 30-61) in 2011 (Cagnazzi 2010, 2013).

Snubfin dolphins were found only north of Port Alma; social structure analysis did not provide any evidence of community structure in the population (Cagnazzi 2011, Cagnazzi et al. 2013c). Analysis of the distribution of sightings indicates that this is the southernmost population of snubfin dolphins in eastern Australian waters and is geographically isolated from conspecific populations outside the region (Cagnazzi et al. 2013c). This hypothesis was supported by preliminary genetic evidence based on very few samples (n = 9), which suggested that snubfin dolphins in Port Alma should be considered as a genetically discrete unit for further actions regarding their conservation and management (Cagnazzi 2011). Total population estimates between 2007–2011 indicated that 105 individuals (SE = 2.5, 95%CI = 100–110) used Port Alma every year over a five year period (Cagnazzi et al. 2013c).

Previous toxicology tests showed that total polycyclic aromatic hydrocarbons (PAHs) concentrations in inshore dolphins from Port Alma and Port Curtis were higher than total PAHs concentrations recorded in humpback dolphins from highly polluted regions such as Hong Kong,

Xiamen and Zhuhai, southern China (Cagnazzi et al. 2013a). Concentrations of dichlorodiphenylethanes (DDTs) and hexachlorobenzene (HCB) were found at levels not considered dangerous to dolphin health. Concentrations of polychlorinated biphenyls (PCBs) in all samples from Port Curtis and in a few samples from Port Alma were within ranges potentially causing immune system suppression as well as reproductive impairment (Kannan et al. 2000, Schwacke et al. 2002, Jepson et al. 2005, Cagnazzi et al. 2013a).

Lower population estimates were reported for humpback dolphins in 2011 compared to previous years suggesting that the abundance in the Port Alma and Port Curtis region may have declined following the 2010–2011 flood, Cyclone Yasi (February 2011) and the start of the WBDDP (March 2011) (Cagnazzi 2013). However, in such relatively small populations only detailed long-term research and monitoring provides sufficient robust data to assess whether such declines are only a temporary shift in the distribution, or representative of a significant permanent loss from the populations (Parra et al. 2006a, Taylor et al. 2007, Hawkins et al. 2017). For example, with the precision of the abundance estimates similar to those obtained in this study (RSE ~ 0.2), it was estimated that it will take between 10 to 15 years to detect a population change of 2 to 4% p.a..

The availability of pre-existing data combined with data collected as part of this study allowed inferences to be made regarding population trends and assessments of the potential effects of the flood and WBDDP on the conservation status of inshore snubfin and humpback dolphins in the ERMP survey area.

2 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.

2.1 Introduction

Obtaining accurate and precise estimates of the abundance of cetaceans ($\widehat{N}$) is usually difficult, expensive and time consuming (Taylor et al. 2007). The precision of the estimates is positively correlated to the population size, capture probabilities (*p*) and survey effort. Therefore, obtaining accurate population estimates is challenging for elusive species living in small populations such as the humpback and snubfin dolphins. Additionally, in populations of small size, higher precision is required to statistically detect trends. The probability of statistically detecting a trend increases with the rate of change, the precision of the estimates, and the length of the samples (Gerrodette 1987). However, even a small decline can cause serious concern for the long-term survival of the small populations (Parra et al. 2006a).

The power to detect a trend increases substantially as the relative standard error of the estimates (RSE($\widehat{N}$) = SE($\widehat{N}$)/$\widehat{N}$; SE$\left(\widehat{N}\right)$ = standard error of the abundance estimates) decreases below 0.2. For example, a RSE of 0.1 has been estimated to detect a 4% rate of change over a seven years study period (assuming annual surveys), whereas a RSE of > 0.2 has been estimated to detect the same trend over a 15 years study period (Parra et al. 2006a). Brooks et al. (2014) suggested that a RSE of the abundance estimates of 0.2 or less is the minimum reasonable criterion for study precision. Since the total number of dolphins present in one region is a fixed parameter, the reliability of abundance estimates depends on obtaining an adequate sample size of individuals and high capture probabilities (White et al. 1982, Menkens and Anderson 1988). Simulation studies have shown that the overall capture probability of the sampled individuals should be greater than 0.1 (greater than 10% of the population should be captured during any occasion) to obtain reliable abundance estimates (White et al. 1982). The survey design used in this study was developed to meet these criteria required to obtain robust and precise abundance estimates ($p > 0.1$ and RSE < 0.2) while following the best practice protocols suggested by the Australian Inshore Dolphin Research Framework (Brooks et al. 2014, Department of the Environment 2015).

2.2 Methods

2.2.1 Model selection

The choice of an appropriate mark-recapture model is fundamentally important to obtaining unbiased and accurate abundance estimates for inshore dolphins. Two general types of models (closed and open population models) are used to estimate abundance and other demographic parameters from mark-recapture data collected over multiple sampling periods. Closed population models assume that the population remains unchanged for the duration of the study (i.e. no gains through births or immigration, nor losses through deaths or emigration), whereas open population models allow for demographic changes in the population over time including gains (births, immigration) and losses (mortality, emigration) (Brooks and Pollock 2011).

Closed models are applied to short-term studies and can accommodate and explicitly model variation in capture probabilities by sampling occasion (time), individual animal response (heterogeneity) and behavioural response to first capture ('behaviour', 'trap happy' and 'trap shy' responses) (Otis et al. 1978). Un-modelled individual heterogeneity biases abundance estimates downward, and un-modelled behavioural response to first capture biases abundance estimates downward if animals become easier to capture ('trap happy') or upward if they became harder to capture ('trap shy') following their first capture.

Open-population models are applied to longer studies and provide an abundance estimate at each sampling occasion (except the first and last unless a reduced parameter model is fitted) as well as the probability of apparent survival (alive and remaining in the sampling area) (Lebreton et al. 1992) and apparent births (born or immigrated) between sampling occasions (Jolly 1965, Seber 1965, Arnason and Schwarz 1996). In contrast to closed models, open models cannot accommodate variation in capture probabilities except by time, and may produce biased abundance estimates in the presence of individual heterogeneity or behavioural response to first capture.

The population model selected for this study was the Closed Robust Design Model (CRDM) (Pollock 1982), which combines the advantage of closed and open population models in a single design. The CRDM incorporates a series of primary periods that are separated by time scales that allow gains and losses from the population. Each primary period is composed of a set of temporally closed secondary periods during which it is biologically acceptable to consider the population closed to unknown changes. Under this model, abundance can be accurately estimated for each primary sampling period in the presence of heterogeneity, and apparent survival can be estimated between primary sampling periods. Additionally, the CRDM can account for temporary emigration which is a potential source of bias in the estimate of survival parameters (Kendall and Nichols 1995, Kendall et al. 1997). The CRDM has been suggested by the Australian Inshore Dolphin

Research Framework as the most appropriate to model inshore dolphin population demographic parameters from data collected during intensive studies (Brooks and Pollock 2011) and it is currently used in several on-going projects on inshore dolphins around Australia (Nicholson et al. 2012, Brooks et al. 2017, Hunt et al. 2017, Passadore et al. 2017).

2.2.2 Defining the number of capture occasions

The simulations facility in the program MARK (White et al. 2002) was used to investigate the relationship between the precision of abundance estimates and the number of secondary periods under different capture probabilities; outcomes of the simulation can be seen in Figures 2a and 2b. These plots were based on sets of 200 simulations for two, three, four, five and six secondary periods with capture probabilities varying from 0.075 to 0.5. Simulations were run for both species separately using prior information on abundance estimates of adult marked humpback dolphins ($\widehat{N}_{marked}$ = 105) and snubfin dolphins ($\widehat{N}_{marked}$ = 77) in Port Alma (Cagnazzi 2013, Cagnazzi et al. 2013b).

Based on these simulations, capture probabilities of more than 0.5 (50% of the marked population must be photographed with excellent quality images) are needed with only two secondary periods (Figure 2a) to obtain abundance estimates of marked adult snubfin dolphins with a RSE of approximately 0.2 (our target of precision). To obtain the same precision of the estimates (RSE = 0.2) with four and five secondary occasions, capture probabilities dropped to 0.3 and 0.2 respectively (Figure 2a). This means that at least 37 adult dolphins of both species must be captured in each secondary period to obtain abundance estimates with a RSE of less than 0.2 with two primary periods. With three, four or five secondary periods the number of marked dolphins to be captured dropped to 26, 23 and 15 respectively. Because the population of marked adult humpback dolphins was larger for the same number of occasions, a higher precision was achieved with lower capture probabilities (Figure 2b). Therefore, the development of the survey design was based on the snubfin dolphin simulation results for $\widehat{N}_{marked}$ = 77, which are displayed in Figure 2a.

Spotting humpback and snubfin dolphins in the wild is particularly difficult as result of their shy behaviour, low profile on the water, and because they are generally found in low densities. Therefore, a capture probability of 0.3 or higher (30% of the marked population) was considered extremely difficult to achieve in one secondary period. Whereas, abundance estimates will have little precision (RSE > 0.4) with capture probability of 0.1, irrespective of the number of capture occasions (Figure 2b). Capture probabilities of ~ 0.2 were therefore considered to be realistic sampling targets. Based on these simulations, the sampling design for the ERMP survey area was

based on CRDM model consisting of five capture occasions per year (secondary periods) replicated for three years (primary periods).

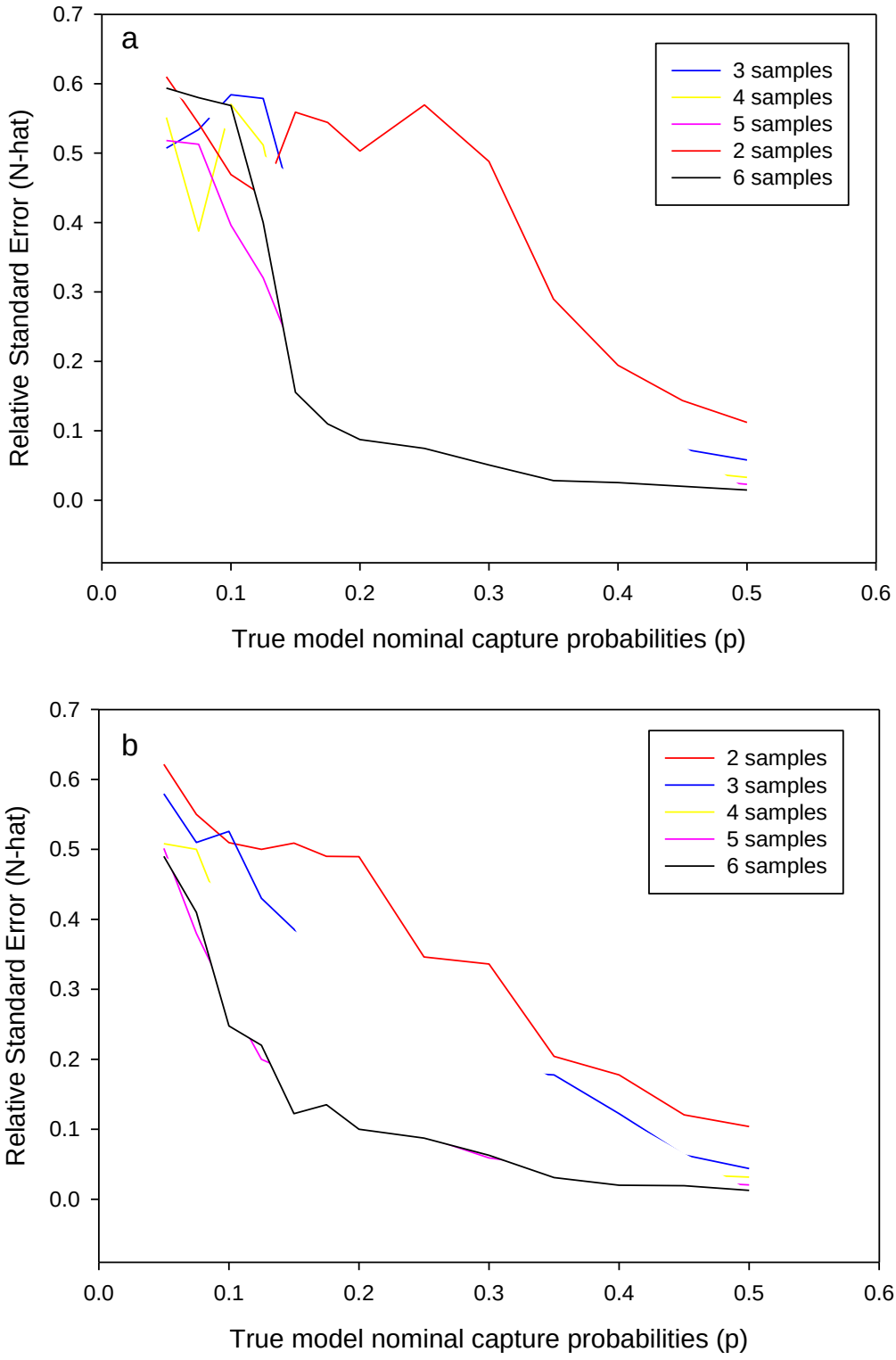

Figure 2. Estimated relative standard errors for a closed model abundance estimate with two, three, four, five and six samples for a) snubfin ($\widehat{N}_{marked}$ = 77) and b) humpback ($\widehat{N}_{marked}$ = 105) dolphins.

2.2.3 Survey Design

In mark-recapture studies, good survey designs should guarantee a uniform and unbiased coverage of the study area to allow homogenous capture probabilities among individuals. A good survey design should also cover a proportion of the study area large enough to guarantee high capture probabilities. Finally, the extent of the area to survey must be realistically achievable within the timeframe required to validate the model assumptions.

Theoretically, a distribution of dolphins is assumed to be uniform throughout the study area, and if all the dolphins present in the sample area are spotted during the surveys, a uniform coverage of 20% of the study area would guarantee the capture of 20% of the marked population ($p = 0.2$). However, dolphins are not uniformly distributed and not all the dolphins are available to be captured. Some individuals may avoid the research vessel, others may be out of the study area during the survey, or underwater and not visible. Therefore, a larger proportion of the study area must be surveyed to reach the defined capture target.

A standardised survey design including strip and transect lines was applied to guarantee a uniform and unbiased coverage of the ERMP survey area (Thomas et al. 2007). Due to the complex topography of the ERMP survey area and to implement the best survey design, the ERMP survey area was subdivided into two sections named based on the map colours “blue-areas” and “brown-areas” (Figure 3). Blue-areas included sufficiently large body of waters where transects lines could be implemented successfully. Brown-areas were mostly creeks or rivers, with the exception being the open coastline on the east side of Curtis Island, with average width small enough (about 1 km at high tide) to guarantee that all animals within that area had the potential to be sighted.

The software Distance (Thomas et al. 2010), which implements automated survey design algorithms (Strindberg and Buckland 2004), was used to design the transects within blue-areas. Initially a grid of points, over which coverage probability was assessed, was generated. Then a new design was created specifying the sampler type (line), design class (parallel), and the strip width (800 m). Within blue-areas, boat-based surveys were conducted following a parallel-stratified sampling design. The parallel line transects were chosen instead of more commonly applied zig-zag survey methods because the ERMP survey area is small enough to allow transects to be placed close together, which minimises the transit time (off-effort time) between transects (Strindberg and Buckland 2004). To survey brown-areas, the research vessel travelled in the centre of the creek, river or channel (transects were adapted based on tide conditions, as most of these areas may not be navigable at low tide), assuming that all the dolphins from the boat line to the edge of the area could be seen (proportion of the area coverage = 1). The open coastline on the east coast of Curtis Island

was classified as a brown-area as it is about 1 km wide and therefore too narrow to fit transect lines (Figure 3).

The total extent of the ERMP survey area was estimated to be 1147 km^2 (blue-areas = 980 km^2; brown-areas = 167 km^2). The survey design was based on two boats operating concurrently, for approximately five days (based on local weather forecast patterns), for an average of 50 km/day (minimum distance covered in one day during previous years). The best design for the blue-areas was obtained by placing transect lines two km apart with a constant half strip width of 400 m (Figure 3), but with different angles per stratum to optimise the survey time. The total transect length for this design was 789 km, for a total coverage corresponding to 45% (area coverage = 460.39 km^2) of the entire blue-area (Figure 3). Using the design above, the total area covered including blue- and brown-areas was 55% (627 km^2) of the entire ERMP survey area. This corresponded to more than double the minimum area to survey in one secondary period that would guarantee the capture of at least 20% of marked dolphins under the assumption of a uniform distribution.

At a speed of 12 km/h, a maximum of 32.87 h per boat were needed to survey all transect lines and brown-areas once (one secondary period). As a result, it was estimated that with two boats operating at the same time, less than six hours per boat per day were needed to complete one secondary period in five days.

Surveys were extended out of the ERMP survey area to Keppel Sands. Keppel Sands is a small area (~ 140 km^2) north of Port Alma (Figure 3) known from previous studies to comparise part of the daily home range of the snubfin and humpback dolphins living in Port Alma (Cagnazzi 2011, Cagnazzi et al. 2013c). The total transect length required to cover this area was estimated to be 71 km, corresponding to 6 h of survey effort.

Under the CRDM, the coverage of the entire ERMP survey area and Keppel Sands once each month was a secondary period, whereas the combination of five full surveys between May and September was a primary period. The final model consisted of three primary periods (May to September of 2014, 2015, 2016), each consisting of five secondary periods.

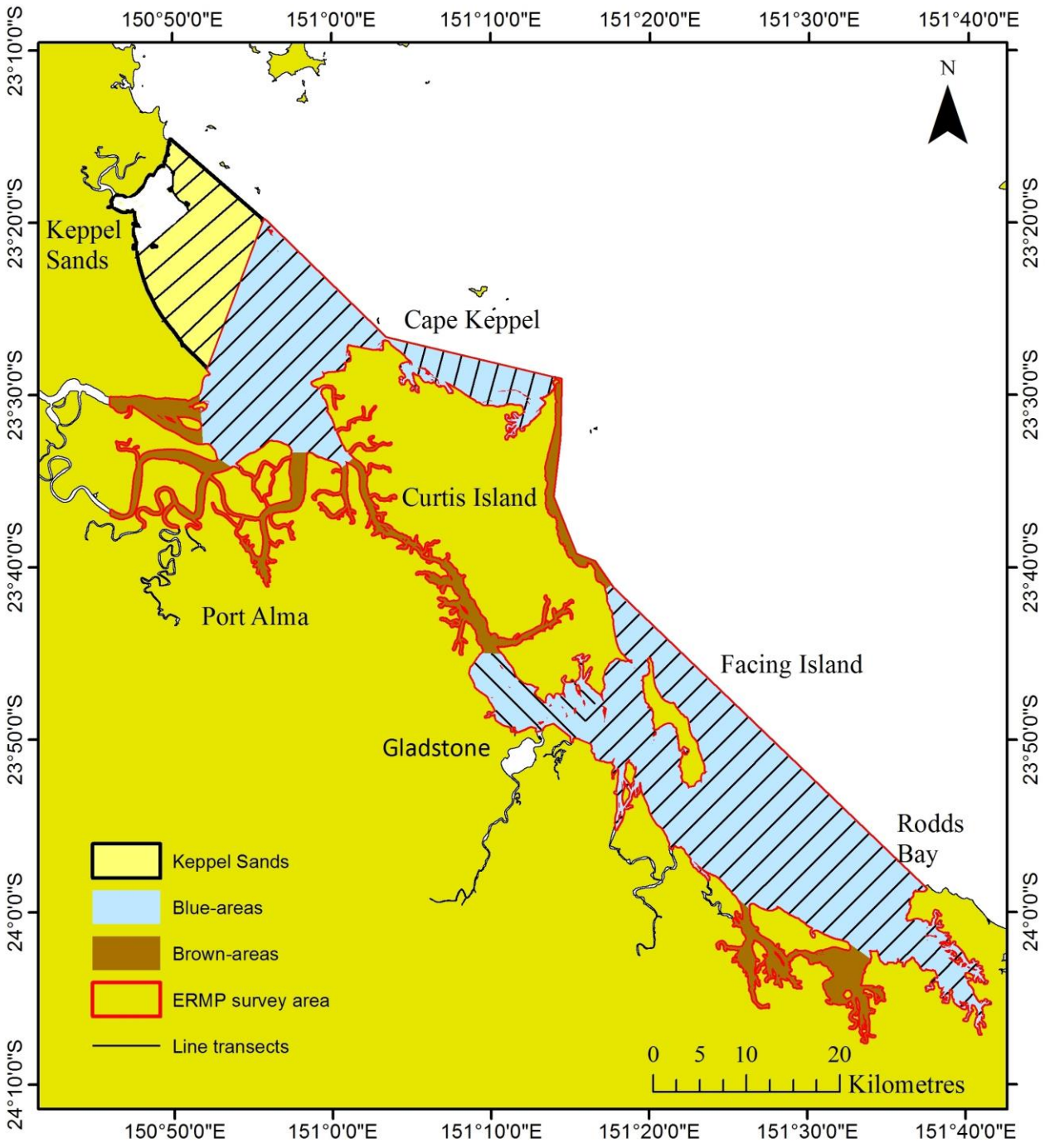

Figure 3 Map of the ERMP survey area and Keppel Sands. Surveys in blue-areas, were conducted following pre-determined line transects 2 km apart (shown in black); blue-area were subdivided into different smaller sub-areas for optimal transect placement. Brown-areas were surveyed as strip transects.

2.2.4 Data collection

The ERMP survey area was divided into four distinct sub areas (Figure 4): Port Alma (424 km^2), Port Curtis (474 km^2), Rodds Bay (211 km^2) and Curtis Island east coast (100 km^2). Keppel Sands, the fifth sub-area, measured ~ 140 km^2. Data collected in Keppel Sands were grouped together with

data collected from of Port Alma for the purpose of the photo-identification mark-recapture analysis.

Port Alma and Port Curtis are separated by the Ramsay Crossing, a very shallow section in the middle of The Narrows that is navigable only during high tide (Figure 4). Port Curtis and Rodds Bay are separated by Seal Rock reef, a rock formation that extends in a straight line from south of the Boyne River to the level of Facing Island (Figure 4). These areas are characterised not only by different ecological habitats but various economic values and human uses. Port Alma form part of the Fitzroy River estuary system; here the coastline has been largely modified primarily for livestock (GBRMPA 2013). Keppel Sands is part of the Fitzroy River estuary. Port Curtis is a naturally sheltered harbour, protected by Facing Island and Curtis Island on the east, which harbours one of Australia's leading ports, utilised particularly for the export of coal (Windle et al. 2017). Rodds Bay is a north facing shallow bay with minimal freshwater input and anthropogenic influence. Curtis Island is an unpopulated open coastline characterised by long sandy beaches punctuated by occasional rock formations and coral reefs.

Data were collected following standard procedures applied in mark-recapture studies focused on inshore dolphins (Parra et al. 2006a, Cagnazzi et al. 2011a). Transects were surveyed using two research vessels at a speed of 12 km/h. Two observers positioned one on each side of the vessel searched for dolphin groups along each transect.

A group was defined as any dolphins within visual distance involved in similar behavioural activities or clearly interacting. After a group was sighted, the research team approached cautiously the group and attempted to take at least one good quality photograph of either the right or left side of the dorsal fin of each individual in the group. Dorsal fin images were taken using either a Nikon or a Canon digital camera equipped with an 80–400 mm and 400 mm fixed zoom lens. Data recorded at each sighting included: species, group size, group composition (adult, juveniles and calves), date, time, geographical location (latitude and longitude) water depth, and behaviour (feeding, travelling, socialising and milling).

Age classes were distinguished based on the size and colour patterns (Figure 5). For both species, individuals of between 2–3 m in length were classified as adults. In humpback dolphins, older adults show whitening of the dorsal fin, rostrum, and body surface while younger adults are of a uniform dark grey skin colour, whereas adults snubfin dolphins are of a dark brown colour. In humpback dolphins, individuals less than two-thirds of an adult body size and of a uniform light grey skin were classified as juveniles. In snubfin dolphins, juveniles were not distinguished from calves. Calves were individuals of less than half of the adult body length and were always associated with an adult, likely to be the mother. Humpback dolphin calves were of a light grey or

black colour, whereas snubfin dolphin calves were light brown. General data collected for each survey included date, start and end times, wind direction and speed, sea state (0 = mostly flat conditions; 1 = few ripples but no white caps, 2 = more consistent ripples with few white caps developing, 3 = extended white caps), visibility (good = sunny conditions with no glare, medium = cloudy condition or glare, poor = visibility largely affected by the combination of various factors), and total transect kilometres surveyed during the day. Data collection was interrupted with a sea state of 3, and/or in the event of poor visibility.

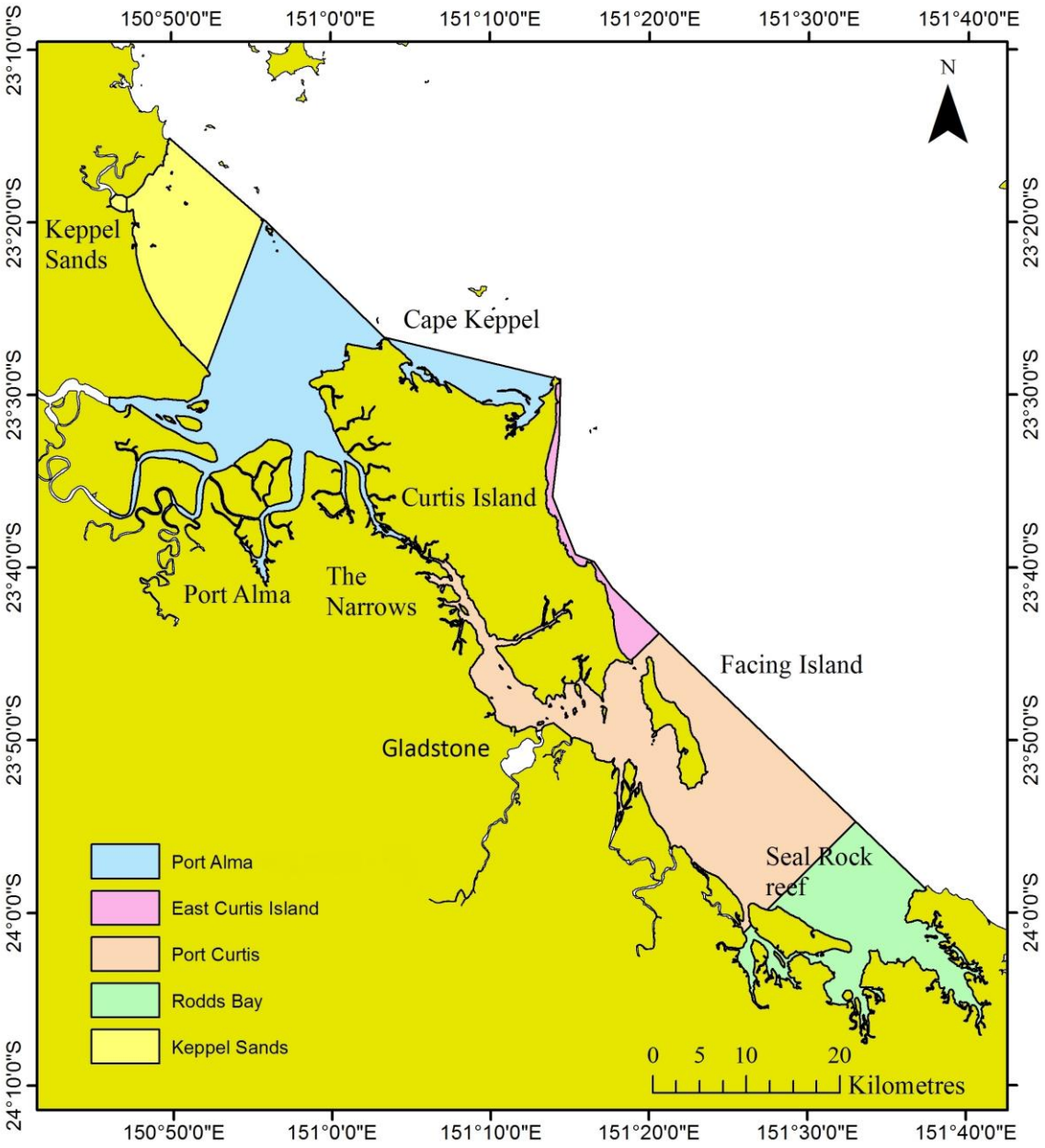

Figure 4. Map of the ERMP survey area divided by sub areas: Port Curtis, Port Alma, Rodds Bay and Curtis Island. Keppel Sands (the small area to the north of Port Alma) was added to the ERMP survey area as it is a known part of the daily home range of the snubfin and humpback dolphins living in Port Alma.

Figure 5 Variation in coloration patterns in adults, juveniles and calves humpback and snubfin dolphins.

2.2.5 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 normally fairly stable over time, allowing for the identification of dolphins from pictures taken from either side of the dorsal fin. Other permanent marks, such as mottled patches, were used as secondary identification marks. Scratches and skin disorders are likely to appear and disappear over time periods less than the study duration hence were not used for primary identification purposes.

All photographs were first categorised by groups sighted during the day, and within each group folder by the name of the person that took the photos, to avoid the risk of a photographer scoring

their own pictures. All images clearly unsuitable for matching purposes were removed before rigorous assessments of image quality for matching were made. The following properties rendered images unsuitable for matching a) with water only, b) with the fin hardly visible in distance, c) evidently out of focus, d) with less than 45% of the fin visible (not showing the entire trailing edge and at least the top half of the leading edge) and e) with dolphins facing directly away or toward the camera. The remaining images were graded according to photographic quality and distinctiveness in order to minimise the introduction of bias and to reduce misidentification (Gowans and Whitehead 2001). All images were assigned an absolute value based on focus (2 = in focus, 4 = slightly off focus, or 9 = off focus), degree of contrast (1 = dorsal fin clearly distinguishable from the background, 2 = dorsal fin partially distinguishable, and 3 = lack of contrast), angle of dorsal fin to the camera (1 ~ 90º, 2 < 135º or 8 ≥ 135º), dorsal fin visibility and the proportion of the frame filled by the dorsal fin (1 = picture quality not affected by pixel resolution, 5 = pixels visible in the picture) (Brooks and Pollock 2011, Tyne et al. 2014). These values were then summed to produce an overall image quality score. All images with a quality score <17 (maximum of one poor parameter) were added to the database and divided into the following categories: “excellent” with a total score of 5–7, “good” with a score of 8–11 and “fair” with a score of 12–17. All photographs with a quality score of > 17 were classified as “poor” and not considered suitable for matching purposes.

Within a group folder all images were divided by individual dolphins. For each individual, the image with the best quality score was uploaded and matched in a web-based catalogue with an automated matching system (www.capricorncetaceansproject.com). The first time a dorsal fin was added to the catalogue it received a distinctiveness value: D-0 not distinctive (none or very little information, very small not clearly distinguishable nicks on trailing edge), D-1 very little distinctiveness with only one clear matching feature, D-2 moderately distinctive dorsal fin with at least two salient matching features, and D-3 highly distinctive dorsal fin with a minimum of three clearly visible features, or two if one was on the leading edge of the dorsal fin (Figure 6). Non-marked adult dolphins were classified as 0 in the database. Photographs of dolphins classified as calves or juveniles were also uploaded to the daily sighting data, but only those with at least a D-2 distinctiveness value were matched against the database and were given a unique identified number. Dorsal fins of calves and juveniles with D-1 or D-0 distinctiveness values were catalogued in the database with a common identifier associated to the age class as -1 and -2 respectively. This information was used to estimate the proportion of non-adult dolphins in the population.

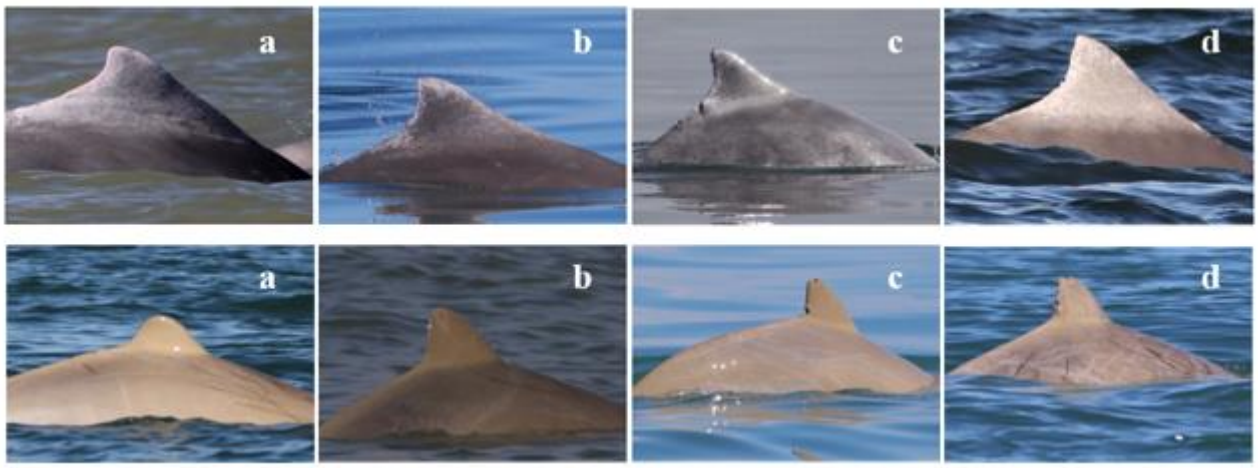

Figure 6 Examples of humpback (top) and snubfin (bottom) dolphins classified using the distinctiveness categories: (a) D-0 no nicks, notches on the leading or trailing edge features or any distinguishable feature, (b) D-1 very little distinctiveness, very small not clearly distinguishable nicks on leading or trailing edge, mottled patches and distinctive shape of the dorsal fin, (c) D-2 two features or one major feature on dorsal fin, and (d) D-3 at least three major features on dorsal fin, or one feature on the leading and one on the trailing edge.

2.2.6 Statistical methods

The CRDM was implemented to estimate abundance, apparent survival and temporary emigration rates (Smith et al. 2013, Brooks et al. 2017).

The mark-recapture models based on a Robust Design make the following assumptions:

1) Natural marks are distinct enough for individual identification without error

2) Capture probabilities between individuals within a sampling event are homogenous (i.e. no heterogeneity and no trap response or un-modelled heterogeneity)

3) Survival probabilities are homogeneous between primary periods

4) Instantaneous sampling for secondary periods

5) Population is closed within primary periods

6) Captures are independent between individuals (clustering causes over dispersion).

The use and reliability of marks (Assumption 1) on the dorsal fin to unequivocally identify and distinguish individuals over time is well established in the vast scientific literature on mark-recapture studies (Smith et al. 2013). Similarly, photo-identification mark-recapture protocols are known to cause no behavioural response to the first capture (Assumption 2) (Nicholson et al. 2012). Furthermore, the systematic sampling design applied in this study reduced the risk of heterogeneity in capture probability due to uneven coverage of the ERMP survey area (Assumption 2). The rigorous image classification process applied in this study minimised the risk of mis-identification

due to low dorsal fin distinctiveness and photo quality (Assumption 2). Any remaining latent heterogeneity was investigated in the modelling process. As survival rate varies with age, only adult individuals were included in the analysis to ensure homogeneous survival probability (Assumption 3). When possible, the sex (male, female, unknown) was included in the modelling process as an individual covariate to test its effect on capture and survival probabilities. To achieve instantaneous sampling and demographic closure (Assumptions 4 and 5), efforts were made to complete each secondary period within a primary period as close together in time as possible. The assumption of population closure within each primary period was investigated using the Stanley and Burnham (1999) and Otis et al. (1978) closure tests, as implemented in the computer program Close Test (Stanley and Burnham 1999). The two tests have been developed on different null hypotheses and, if used in conjunction, allow for better detection and interpretation of closure violations in mark-recapture datasets. The Stanley and Burnham closure test allows for time variation in capture probabilities in the absence of behavioural responses and heterogeneity. The Otis et al. (1978) closure test allows for investigation of population closure in the presence of heterogeneity in capture probabilities.

Finally, the assumption of independence in captures (Assumption 6) is always violated for dolphins because they occur in clusters or local populations, which causes data over-dispersion. The goodness of fit test (GOF test) available in the program U-CARE (Choquet et al. 2005) was used to test data over-dispersion. To do this, secondary periods within primary periods were collapsed into a single occasion to meet the format of a Cormack-Jolly-Seber model (Lebreton et al. 1992).

2.2.7 Alignment of data collection with the design's requirements: capture probability, abundance estimates, and relative standard error

The program MARK was employed to fit the full likelihood parameterisation of the CRDM. The models consisted of three primary periods, each composed of five secondary periods (paragraph 2.2.2). Intervals between primary periods were specified in decimal years between their final dates to obtain consistent, per annum estimates of apparent survival.

The parameters estimated by the model included probability of first capture (p) and recapture (c) for each secondary period, probability of apparent survival (ϕ), and two temporary emigration parameters for each interval between primary sampling periods. The two temporary emigration parameters estimated the probability of emigration from the ERMP survey area given that the animal was present in the last period [$\gamma''(i)$], and the probability of staying away from the ERMP survey area given that the animal has left the survey area before this period [$\gamma'(i)$]. Finally,

abundance estimates of marked ($\widehat{N}_{marked}$) adults were provided for each primary period as a derived parameter.

The modelling process followed a standard protocol; at first, the most appropriate among the eight Otis et al. (1978) closed population models for each primary period was selected. The Otis et al. (1978) closed population models assume three different sources of variation in encounter probabilities: time (t), behavioural responses to initial capture (b), and inherent differences of individuals, or individual heterogeneity (h). The combination of these three factors leads to a suite of eight models: M_o, M_t, M_b, M_h, M_{bh}, M_{th}, M_{tb}, and M_{tbh}. The model M_0 is the null model with constant detection probabilities. Temporary emigration was modelled as random $\gamma''(i) = \gamma'(i)$, Markovian when $\gamma''(i) \neq \gamma'(i)$, or no temporary emigration $\gamma''(i) = \gamma'(i) = 0$. Apparent survival was the last parameter modelled.

The Akaike Information Criterion for small sample size (AICc) was used to evaluate the relative support for candidate models. Models that produced spurious parameter estimates but had very low AICc values were omitted from the model ranking process. If more than one model provided a reasonable fit to the data (ΔAICc < 2), the weighted model averaging procedure was applied within the program MARK to produce more stable estimates than selecting a single 'best' model from a number of closely-related models (Burnham and Anderson 2002). For each model, the AICc values, AICc weights, model likelihood, numbers of parameters and deviances were also reported. In cases of significant lack of fit (GOF tests *p*-value < 0.05), the model ranking process was adjusted using an estimate of the variance inflation factor. The variance inflation factor was estimated from U-CARE output results as the ratio of the overall test statistic for the model and the model degrees of freedom (Choquet et al. 2005). On these occasions, the quasi-AICc value (QAICc) was used to evaluate model fit (Burnham and Anderson 2002).

The adequacy of the sample regime, capture probabilities and the RSE of the abundance estimates were reported for each best fitting model.

2.2.8 Estimates of total population size

Mark-recapture models yield estimates only for the proportion of marked individuals in the population. However, not all individuals have sufficiently distinctive marks to support unambiguous identification. The total abundance ($\widehat{N}_{total}$) of each population for any sampling period and site was estimated by dividing the estimated abundance of marked dolphins ($\widehat{N}_{marked}$) by the estimated proportion of marked individuals ($\widehat{M}_p$), excluding calves and juveniles.

$\widehat{N}_{tot} = \widehat{N}_{marked}/\widehat{M}_p$.

The proportion of marked individuals of the population was estimated using a mixed effects binary logistic model fitted to the distinctiveness data (1 = distinctively marked, 0 = not distinctively marked) with group, and individuals within group, as random factors (Brooks et al. 2017). Only images with final quality score of < 17 were included in the analysis.
Standard error (SE), the lower ($\widehat{N}_{lower}$) and upper ($\widehat{N}_{upper}$) log-normal confidence intervals for abundance estimates were calculated as:

$$SE(\widehat{N}_{total}) = \sqrt{Var(\widehat{N}_{marked})/(\widehat{N}_{marked})^2 + Var(\widehat{M}_p)/(\widehat{M}_p)^2}.$$

$$\widehat{N}_{lower} = \widehat{N}_{total}/C.$$

$$\widehat{N}_{upper} = \widehat{N}_{total} \times C.$$

$$C = \exp(z_{\alpha/2}\sqrt{\ln\left[1 + \left(SE(\widehat{N}_{total})/\widehat{N}_{total}\right)^2\right]}.$$

2.3 Results

2.3.1 Survey effort

Within the ERMP survey area, a total of 9,383 km of transects were sampled, over three primary periods and 15 secondary periods, covering ~ 80% of the maximum estimated transect length of 11,835 km. During each secondary period, between 61% (487 km) and 93% (736 km) of the estimated maximum (789 km) transect length (Table 1) was sampled. Additionally, during each primary period a minimum of ~ 170 km of transects were run in Keppel Sands (Table 1). Differences between the completed and expected area coverage were due to limitations on sampling capabilities imposed by weather conditions and the inaccessibility of some areas at lower tides (water depth below 0.5). An example of the track covered during one secondary period is shown in Figure 7, and the remaining transects are shown in Figures A.1 to A.3. (Table 1, Figure A.1 to A.3). Each secondary period was completed between a minimum of six to a maximum of 14 days (Table 1). This is a reasonable time period within which we can assume the population is closed but long enough to allow all resident individuals to visit the area and be available for capture.
On average, 8.2 h of visual survey was completed per day (range: 5.2–9.5 h), with the earliest start at 06:01 and the latest end of the survey at 16:55. All surveys were started in a sea state ≤ 1 and paused in presence of extended white caps (or with sea state = 3) to ensure maximum probability of sighting dolphin groups.

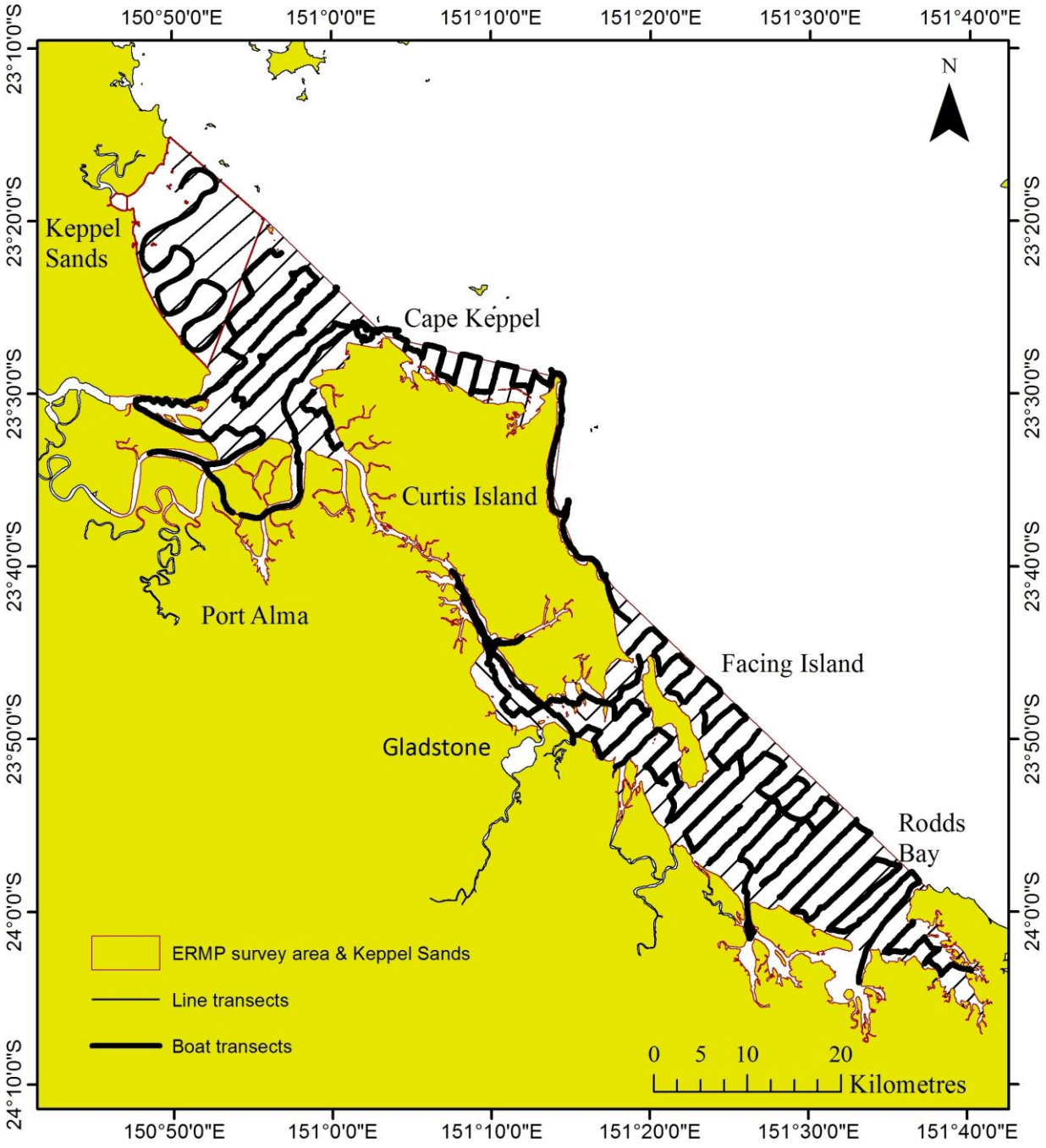

Figure 7 Example of transects covered in the survey areas during one secondary sampling occasion.

Table 1 Study period and effort summary for the 2014-2016 survey seasons divided by primary periods and secondary periods (sp). Survey efforts are expressed for the entire ERMP survey area (only ERMP in the table) and for each sub area (PA = Port Alma, PC = Port Curtis, RB = Rodds Bay, ECI = East Curtis Island).Survey efforts for the Keppel Sands (KS) sub area, which is not part of the ERMP survey area are reported separately. Transects are expressed in km surveyed in one secondary period. Total days = length of period in days needed to survey each area.

Site	Survey Efforts	Primary period 1[a]					Primary period 2[b]					Primary period 3[c]				
		sp1	sp2	sp3	sp4	sp5	sp1	sp2	sp3	sp4	sp5	sp1	sp2	sp3	sp4	sp5
PA	Transects	235	219	213	220	203	183	303	227	220	209	318	227	247	217	242
	Total days	3	9	5	3	3	11	4	9	12	8	1	12	7	4	6
PC	Transects	211	209	246	230	223	292	265	299	230	248	292	253	271	232	227
	Total days	6	4	1	3	1	1	1	2	6	6	3	1	2	2	5
RB	Transects	40	88	91	87	78	98	102	113	87	91	85	97	90	128	113
	Total days	1	1	1	1	1	1	2	1	1	1	1	1	1	2	1
ECI	Transects	0	0	67	36	80	86	0	40	88	46	40	44	90	36	46
	Total days	0	0	2	1	2	3	0	1	1	2	2	1	2	1	1
ERMP	Transects	487	517	617	574	586	660	672	681	626	596	736	622	699	614	628
	Total days	11	11	8	9	7	12	8	10	12	11	6	14	12	4	7
KS	Transects	0	45	65	65	48	35	49	59	58	0	45	33	33	38	37
	Total days	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1

[a] 26 May to 9 September 2014; [b] 23 May to 8 September 2015; [c] 22 May to 19 September 2016

2.3.2 Summary of photo-identification data

A total of 249 groups (with at least one photograph collected) of humpback dolphins, 122 groups of snubfin dolphins (Table 2, Figure 8 and Figure 9) and 15 groups of bottlenose dolphins (*Tursiops aduncus*) were encountered in the ERMP survey area during this study. Bottlenose dolphins were not considered further in the analysis due to the low number of sightings. Average group size estimates (Table 3) were very similar between species and across sites and years. The larger aggregations of humpback dolphins with estimated group sizes ranging between 16 and 29 individuals were all sighted in Port Curtis.

A total of 17,808 images were considered acceptable for photo-identification purposes for both species in 2014 (humpback = 13,351; snubfin = 4,457), 14,393 images were acceptable in 2015 (humpback = 8,560; snubfin = 5,833) and 14,160 images in 2016 (humpback = 11,073; snubfin = 3,087). Of these, 32,381 images received scores below minimum quality criteria ($Q \leq 17$) and were not included in the database. By combining high quality images of left and/or right sides of dorsal fins of individual dolphins, a total of 181 adult humpback dolphins (distinctiveness scores: D-1 = 45, D-2 = 81, D-3 = 55) and 127 adult snubfin dolphins (distinctiveness scores: D-1 = 25, D-2 = 43, D-3 = 59) were catalogued. All marked adult individuals (i.e. with distinctiveness scores D-1 to D-3) were included in the analysis to maximize capture probabilities. The distinctiveness score was included into the the modeling process as covariate to test the effect on capture probabilities.

Of the 181 individual humpback dolphins identified, 128 were confirmed as female, 14 as male and 39 were of unknown sex. Of the 127 snubfin dolphins identified, 41 were confirmed as female, 21 male and 65 of unknown sex. Sex was determined based on the presence of a dependent calf (humpback = 121, snubfin = 41) genetic analysis (humpback = 53, snubfin = 32).

Table 2 Summary of group sightings by site, species and secondary period. In the table: ERMP = ERMP survey area, PA = Port Alma, KS = Keppel Sands, PC = Port Curtis, RB = Rodds Bay, ECI = East Curtis Island, hd = humpback dolphin, sd = snubfin dolphin, primary periods = pp, secondary periods = sp.

Study sites & Species		**pp 2014 = 1**					**pp 2015 = 2**					**pp 2016 = 3**				
		sp 1	**sp 2**	**sp 3**	**sp 4**	**sp 5**	**sp 1**	**sp 2**	**sp 3**	**sp 4**	**sp 5**	**sp 1**	**sp 2**	**sp 3**	**sp 4**	**sp 5**
ERMP	hd	16	14	20	17	16	13	15	18	20	23	9	12	25	13	18
	sd	6	8	6	4	4	9	2	9	24	13	10	9	6	4	8
PA+KS	hd	3	5	8	8	0	5	6	9	7	7	3	3	2	2	4
	sd	6	8	6	4	4	9	2	4	24	13	10	9	6	4	8
PC	hd	13	7	11	9	11	7	6	12	9	11	5	8	12	4	5
	sd	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ECI	hd	0	0	0	0	2	0	0	0	2	0	0	0	3	0	0
	sd	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RB	hd	0	2	1	0	3	1	3	3	2	5	1	1	8	7	9
	sd	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3 Average group size ± standard error (Mean±SE) estimated for humpback dolphins (hd) and snubfin dolphins (sd) in the ERMP survey area (ERMP), Port Alma (PA), Keppel Sands (KS), Port Curtis (PC), East Curtis Island (ECI) and Rodds Bay (RB).

Primary periods		**2014 = 1**			**2015 = 2**			**2016 = 3**		
Species	**Site**	**n**	**Mean±SE**	**Range**	**n**	**Mean±SE**	**Range**	**n**	**Mean±SE**	**Range**
sd	ERMP	25	4.4±0.66	1–13	57	4.2±0.57	1–19	40	3.9±0.42	1–13
hd	ERMP	83	5.8±0.49	1–25	88	5.4±0.52	1–29	78	5.2±0.48	1–26
hd	PA+KS	26	5.1±0.84	1–15	30	4.3±0.72	1–16	14	4.3±0.73	1–12
hd	PC	51	6.1±0.65	1–25	44	5.7±0.84	1–29	38	5.3±0.80	1–26
hd	ECI	2		4–12	2		1–9	0	0	0–0
hd	RB	6	7.1±1.89	1–13	14	4.52±1.2	1–13	25	5.5±0.77	1–15

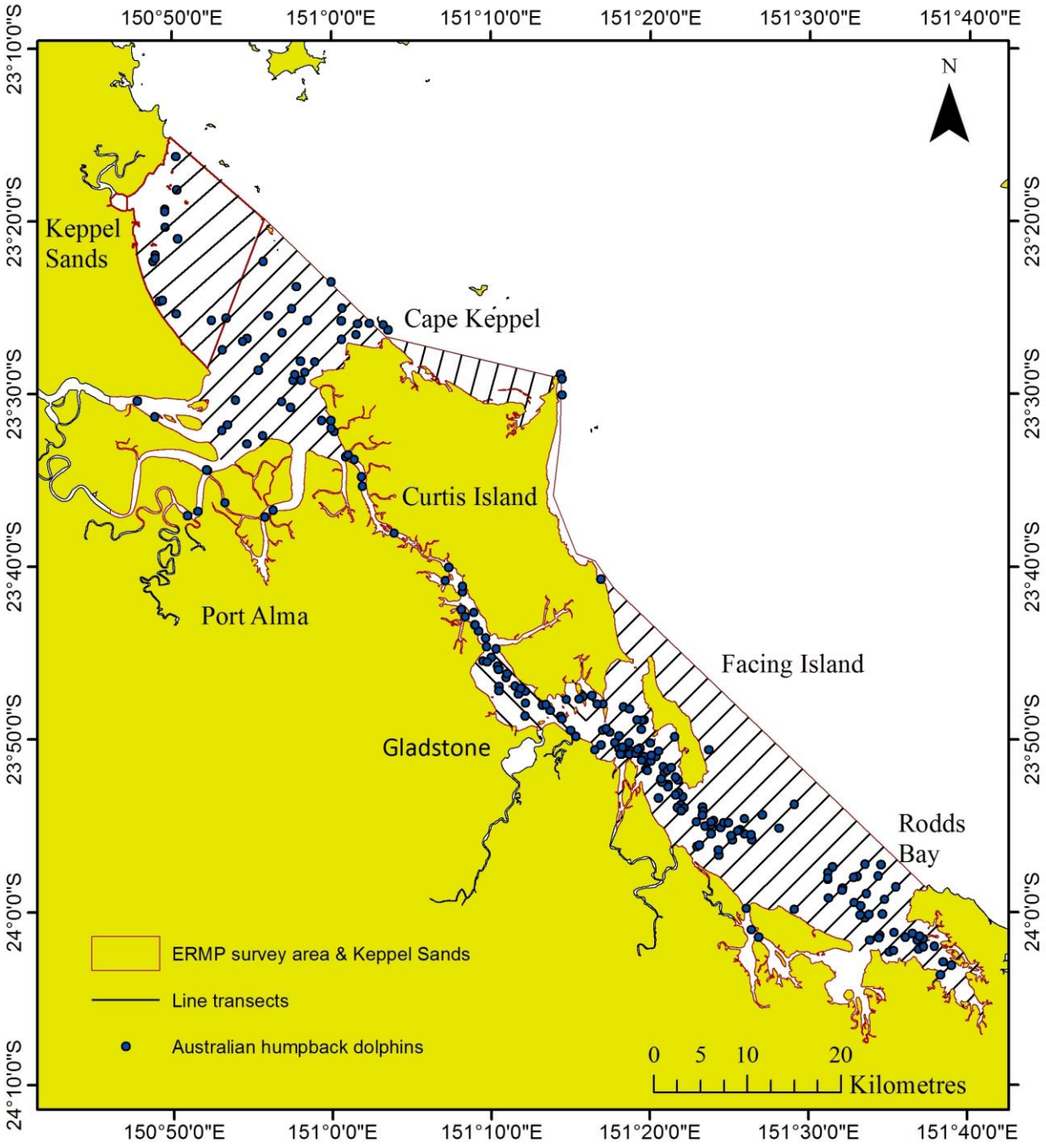

Figure 8 Distribution of humpback dolphin groups sighted in the ERMP survey area and Keppel Sands during boat-based transect surveys between 2014 and 2016.

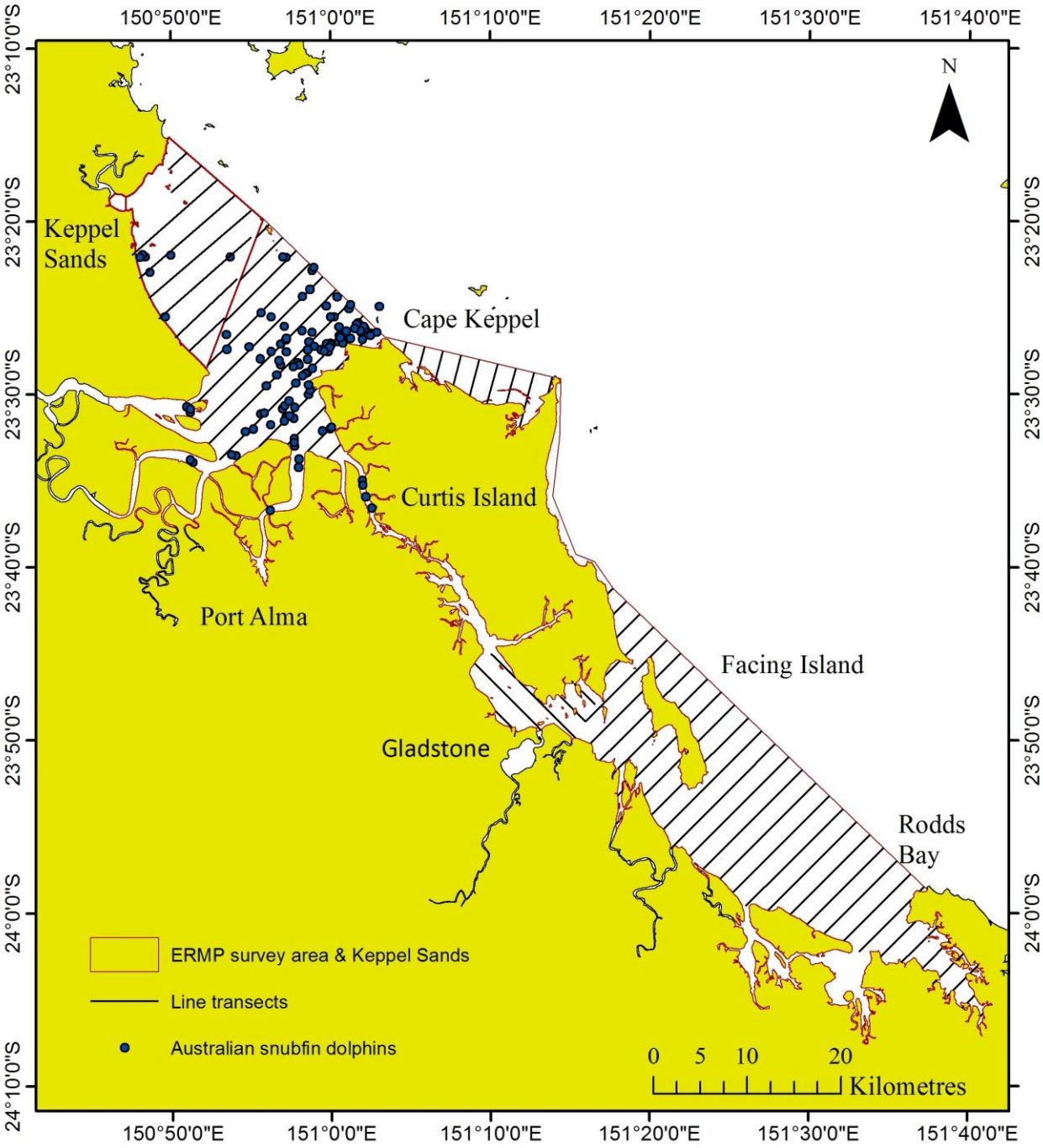

Figure 9 Distribution of snubfin dolphin groups sighted in the ERMP survey area and Keppel Sands during boat-based transect surveys between 2014 and 2016.

2.3.3 Population estimates of Australian snubfin dolphins

Snubfin dolphins were confirmed to occur only in Port Alma. The 127 marked adult dolphins were captured a total of 261 times; 79 individuals (62%) were photographed in only one primary period, 33 (25%) in two, and 15 (12%) in all three primary periods (Table 4).

Both the Stanley and Burnham (1999) and the Otis et al. (1978) closed tests yielded high *p*-values suggesting that the population was closed during each primary period (Table 4). The GOF Test

indicated no data over-dispersion (χ^2 = 0.248; *p*-value = 0.6), therefore no adjustments to variation inflation factor were required.

Eight CRDM were fitted to the snubfin mark–recapture data (Table 5). No useful parameter estimates could be obtained from models including latent heterogeneity and behavioural responses. Models with simplified temporal structures for capture probabilities and distinctiveness as covariates fitted very poorly and yielded unrealistic parameter estimates. We did not attempt to test the effect of sex as a covariate due to the high number of animals (49%) of unknown sex. The two best-fitting models (lowest AICc value) had capture probabilities varying by both primary and secondary period, constant or varying probability of apparent survival, no temporary emigration and accounted for 83% of the total AICc weight (Table 5 and Table A.1).

The proportion of marked individuals (not including juveniles and calves) was estimated to be 0.87 (SE = 0.02). The total abundance of adult snubfin dolphins varied substantially across primary periods from 100 in 2014 (SE = 19, 95%CI = 68–147) to a maximum of 163 in 2015 (SE = 17, 95% CI = 132–200) and down to 103 in 2016 (SE = 17, 95% CI = 73–144) (Table 6). The proportion of juveniles and calves was estimated to be 0.17 (SE = 0.01). The RSE of these estimates (Table 6) was equal to, or below, the target of 0.2 indicated as the maximum reasonable criterion for study precision but above our intended target of 0.1. Capture probabilities were mostly below our target of 20%, and in one secondary period for each primary period also below 10% (Table A.1) required for reliable estimates.

Table 4 Summary of mark-recapture data for snubfin dolphins in the ERMP survey area. In the table n(j) = animals caught, M(j) = total caught, u(j) = newly caught, f(j) = frequencies, SBCS = Stanley and Burnham closure test value, df = degree of freedom, Otis = Otis et al. (1978) closure test value, pp = primary period.

pp	n(j)	M(j)	u(j)	f(j)	SBCS	df	*p*-value	Otis	*p*-value
1	41	47	47	1/79	4.617	5	0.464	-0.316	0.375
2	91	110	63	2/33	12.664	6	0.048	1.914	0.972
3	52	127	17	3/15	5.433	6	0.489	2.410	0.992

Table 5 Mark-recapture models fitted to the capture histories of adult snubfin dolphins including number of parameters estimated (n Par.), apparent survival (ϕ), temporary emigration (γ'', γ') and capture probability (p). M indicates the closed model selected in each primary period, t = p varying with time, b = p varying with behavioural responses and h = latent heterogeneity 2 levels. AICc = Akaike information criterion for small sample size.

Model structure	AICc	ΔAICc	AICc Weights	Model Likel.	n Par.	Deviance
ϕ(.) γ'= γ''(0)Mt	-64.97	0	0.48	1	19	163
ϕ(t) γ'= γ''(0)Mt	-64.39	0.58	0.35	0.74	20	161
ϕ(t) γ'= γ''(.)Mt	-62.01	2.96	0.10	0.22	21	161
ϕ(t) γ'(.)γ''(.)Mt	-59.61	5.36	0.03	0.06	22	161
ϕ(t) γ'(t) γ''(t)Mt	-57.19	7.78	0	0.02	23	161
ϕ(t) γ'(t) γ''(t)Mtb	-56.94	8.03	0	0.01	35	130
ϕ(t) γ'(t) γ''(t)Mth	-36.40	28.57	0	0	41	134
ϕ(t) γ'(t) γ''(t)Mh	-14.93	50.04	0	0	17	217
ϕ(t)γ'(t) γ''(t)Mthb	4.60	69.58	0	0	65	98

Table 6 Estimates of the total marked population size ($\widehat{N}_{marked}$) and total population size ($\widehat{N}_{total}$) of snubfin dolphins in the ERMP survey area, with number of marked dolphins captured (n), lognormal 95% lower and upper confidence intervals (95%CI), standard error (SE) and relative standard error (RSE) for each primary period (pp).

pp	n	$\widehat{N}_{marked}$	SE($\widehat{N}_{marked}$)	95%CI	$\widehat{N}_{total}$	SE($\widehat{N}_{total}$)	RSE	95%CI
1	47	88	17	65–137	100	21	0.20	68–147
2	91	143	15	121–180	163	20	0.17	132–200
3	52	90	15	70–134	103	19	0.18	74–144

2.3.4 Population estimates of Australian humpback dolphins

Humpback dolphins were found throughout the entire ERMP survey area. Of the 181 marked adult humpback dolphins, 53 were sighted in Port Alma and Keppel Sands, 92 in Port Curtis and 65 in Rodds Bay. A large number of dolphins (n = 36) were sighted in both Port Curtis and Rodds Bay, four were sighted in both Port Alma/Keppel Sands and Port Curtis, and one in both Port Alma/Keppel Sands and Rodds Bay. A total of 12 marked adult humpback dolphins were sighted along the eastern side of Curtis Island, all of which were captured only once. Movement across sites occurred mostly within the same primary period, often within secondary periods but also sometimes within the same day. Multistate models assume that movement across sites occurs only between primary periods and therefore could not be applied to these data.

Separate population estimates were generated for: a) ERMP survey area, b) Port Alma/Keppel Sands, c) Port Curtis plus Rodds Bay, and d) Port Curtis alone. Data were not fitted to Rodds Bay because several secondary periods resulted in no captures.

Overall, there was no evidence for violation of the closure assumption in any of the datasets (Table 7). The GOF test indicated significant lack of fit for three datasets (ERMP survey area: χ^2 = 4.112, p-value = 0.04; Port Curtis and Rodds Bay: χ^2 = 5.359, p-value = 0.02; Port Curtis: χ^2 = 6.392, p-

value = 0.01) with the exception of Port Alma/Keppel Sands (χ^2 = 0.147; *p*-value = 0.70) (Table 7). When required, the variation inflation factor was adjusted accordingly to account for data over dispersion.

In all four datasets, the best fitting models had constant apparent survival, no temporary emigration and a different capture probability for each primary and secondary sampling occasion (Table 8 and A.3 to A.6). The proportion of marked individuals was estimated to be 0.92 (SE = 0.01). The proportion of calves and juveniles was estimated to be 0.35 (SE = 0.01). Total abundance estimates for the ERMP survey area were 162 (SE = 9; 95%CI 144–181) in 2014, 162 (SE = 9; 95%CI 144–182) in 2015 and 140 (SE = 10; 95% CI 122–161) in 2016 (Table 9). Average capture probabilities were greater than 0.23 (SE = 0.02) and RSE mostly below 0.07 (Table 9 and A3) which is an indication of very good precision in the results. This model yielded a mean apparent yearly survival estimate for adults of 0.70 (SE = 0.04).

In Port Alma, the total number of humpback dolphins declined sharply from about 68 in 2014 to 36 in 2016, and the estimated average apparent annual survival probability was 0.44 (SE = 0.08, 95% CI = 0.29–0.61) (Table 9 and A6). In Port Curtis and Rodds Bay combined, the number of humpback dolphins slightly increased from 101 in 2014 to 124 in 2015 and 108 in 2016, and the estimated apparent survival was 0.82 (SE = 0.11, 95% CI = 0.50–0.95). The number of humpback dolphins in Port Curtis alone varied across primary periods between a minimum of 67 to a maximum of 85 (Table 9) and the estimate of apparent survival was 0.67 (SE = 0.16, 95% CI = 0.33–0.89). Capture probabilities and RSE were within expected targets, which indicate precise and reliable estimates. Parameter estimates for models of best fit are reported in Tables A3 to A6.

Table 7 Summary of mark-recapture data for humpback dolphins. ERMP = ERMP survey area, PA = Port Alma, KS = Keppel Sands, PC = Port Curtis, RB = Rodds Bay, pp = primary periods, n(j) = animals caught, M(j) = total caught, u(j)= newly caught f(j) = frequencies, SBCS = Stanley and Burnham closure test value, df = degree of freedom, Otis = Otis et al. (1978) closure test value and *p*-values.

Sites	pp	n(j)	M(j)	u(j)	f(j)	SBCS	df	*p*-value	Otis	*p*-value
ERMP+KS	1	120	120	120	75	8.988	6	0.174	-0.010	0.49
	2	118	167	47	59	8.105	6	0.230	2.069	0.98
	3	98	181	14	48	6.481	6	0.371	-1.174	0.12
PA+KS	1	41	41	41	35	10.903	5	0.053	-2.145	0.01
	2	28	50	9	17	9.580	5	0.088	1.906	0.97
	3	21	59	9	7	2.086	3	0.554	NA	NA
PC	1	63	63	63	42	7.307	6	0.293	1.944	0.97
	2	61	87	24	26	1.489	6	0.960	1.652	0.95
	3	48	94	7	26	8.365	4	0.079	-1.336	0.09
PC+RB	1	80	80	80	42	11.780	6	0.067	1.796	0.96
	2	92	121	41	47	3.091	6	0.797	1.141	0.87
	3	78	127	6	38	3.091	6	0.797	1.141	0.87

Table 8 Mark-recapture models fitted to the capture histories of adult humpback dolphins including number of parameters estimated (n Par.), apparent survival (ϕ), temporary emigration (γ'', γ') and capture probability (p). M indicates the closed model selected in each primary period, with t = p varying with time, b = p varying with behavioural responses and h = latent heterogeneity 2 levels. Models were ranked using the quasi-Akaike information criterion for small sample size (QAICc). ERMP = ERMP survey area, PA = Port Alma, KS = Keppel Sands, PC = Port Curtis, RB = Rodds Bay.

Dataset	Model	QAICc	ΔQAICc	QAICc Weight	Model Likel.	n Par.	Deviance
ERMP+ KS	ϕ(.)γ'(.)=γ''(0)Mt	-8.33	0.00	0.64	1.00	19	-47.76
	ϕ(t)γ'(.)=γ''(0)Mt	-6.31	2.01	0.23	0.37	20	-47.90
	ϕ(t) γ'(.)=γ''(.)Mt	-4.18	4.15	0.08	0.13	21	-47.93
	ϕ(t)γ'(t) γ''(t)Mtb	-2.01	6.32	0.03	0.04	22	-47.93
	ϕ(t)γ'(.)γ''(.)Mt	0.16	8.49	0.01	0.01	23	-47.93
	ϕ(t)γ'(t) γ''(t)Mt	0.41	8.74	0.01	0.01	17	-34.74
	ϕ(t)γ'(t) γ''(t)Mth	19.39	27.72	0.00	0.00	35	-55.51
	ϕ(t)γ'(t)γ''(t)Mthb	32.70	41.03	0.00	0.00	41	-56.08
	ϕ(t)γ'(t) γ''(t)Mh	83.58	91.91	0.00	0.00	65	-64.15
PA+KS	ϕ(.)γ'(.)= γ''(0)Mt*	166.83	0	0.61	1	19	122.23
	ϕ(t)γ'(0)γ''(0)Mt*	169.09	2.40	0.18	0.30	20	121.87
PC+RB	ϕ(.)γ'(.)= γ''(0)Mt	33.98	0	0.66	1	19	-5.96
	ϕ(t)γ'(0)γ''(0)Mt	36.17	2.19	0.22	0.33	20	-5.98
PC	ϕ(.)γ'(.)= γ''(0)Mt	53.73	0	0.63	1	19	12.92
	ϕ(t)γ'(0)γ''(0)Mt	56.03	2.30	0.20	0.31	20	12.92

Models with "*" were ranked with the Akaike information criterion for small sample size (AICc). ΔQAICc or ΔAICc < 2 indicates good fit to the data. The notation "." indicates that a given parameter was kept constant and t indicates that a given parameter varies with time. Probability of temporarily emigrating was set as $\gamma' = \gamma'' = 0$ no emigration model, $\gamma' = \gamma''$ random emigration model, $\gamma'(t) \neq \gamma''(t)$ Markovian emigration model.

Table 9 Estimates of the total marked population size ($\widehat{N}_{marked}$) and total population size ($\widehat{N}_{total}$) of humpback dolphins in the ERMP survey area (PA = Port Alma, KS = Keppel Sands, PC = Port Curtis, RB = Rodds Bay), with number of marked dolphins capture (n), lognormal 95% lower and upper confidence intervals (95%CI), standard error (SE) and relative standard error (RSE) for each primary period (pp).

Dataset	pp	n	$\widehat{N}_{marked}$	SE($\widehat{N}_{marked}$)	95%CI	$\widehat{N}_{total}$	SE($\widehat{N}_{total}$)	RSE	95%CI
ERMP+	1	110	149	9	137–171	162	9	0.05	144–181
KS	2	119	150	9	137–172	162	10	0.05	144–182
	3	97	130	9	116–153	140	10	0.07	122–161
PA+KS	1	41	63	10	50–93	68	11	0.16	50–94
	2	29	32	3	29–43	35	3	0.09	29–42
	3	21	33	7	25–56	36	8	0.21	24–55
PC+RB	1	80	94	5	87–109	101	6	0.05	91–114
	2	88	115	8	103–135	124	9	0.07	108–143
	3	78	100	7	90–120	108	8	0.07	94–126
PC	1	63	70	4	66–81	76	4	0.05	68–84
	2	61	78	6	69–96	85	7	0.08	71–99
	3	48	62	6	54–81	68	7	0.10	55–83

2.4 Discussion

2.4.1 Survey effort limitations

Almost all secondary periods were completed using two boats with the exception of the first two secondary occasions in primary occasion one which were completed with one boat only.
During each secondary period attempts were made to survey all transects once. However, full coverage of the study area was never reached. During each secondary period we were able to survey between 61% and 80% of the planned transect length. The difference between effective versus expected coverage was due primarily to the inaccessibility (water depth below 0.5) of some areas at lower tides. Within Port Alma it was estimated that a minimum of 12% of transects (Figure A.4) overlapped with areas exposed or not navigable at low tide (< 0.5 m). A similar or a higher proportion of transects is expected to be inaccessible at low tide in Port Curtis and Rodds Bay. In low tide conditions these areas were not accessible to dolphins and therefore the entire transect was assumed sampled. When transects were left incomplete for reasons others than tidal conditions, attempts were made to survey the remaining section of the planned transect within the same secondary period. About 1.5% of transects were not accessible because the area was reclaimed for human activities.
We were not able to survey East Curtis Island sub-area and the region off Facing Island during each capture occasion due to the remoteness of the region and generally poor sea state conditions. Along East Curtis Island sub-area the sea state was often ≥ 3 even with low winds (Figures A1, A2, A3). Similar limiting factors were encountered off Facing Island where, in some secondary periods, we followed survey protocols applied along East Curtis Island to survey as much possible of the study area with good sighting conditions (Figures A1, A2, A3). Finally, after surveys with sea state conditions ≥ 3 were eliminated from the dataset, Keppel Sands also resulted in no data on two secondary occasions.
Differences in spatial location of sampling may result in heterogeneity in capture probabilities only in case where different groups of dolphins are using different areas. There is no reason to assume a social partitioning of space within the survey area for either species, with relatively many humpback dolphins re-sighted at different sites and snubfin dolphins found only in Port Alma. The reduction from planned survey effort achieved in the study was somewhat greater in inshore than offshore areas, due to inshore areas being more affected by low tides. There is no reason to assume that sub-groups of dolphins use the inshore and offshore areas in different proportion and therefore, differences in sampling effort in the inshore and offshore areas should affect all dolphins equally: i.e., it is unlikely that changes in the ratio of inshore to offshore effort would introduce differences among dolphins in the probability of capture (i.e., individual heterogeneity).

Similarly, since the same group of humpback dolphins uses Port Alma and Keppel Sands to a similar extent (Cagnazzi 2011), uneven coverage of Keppel Sands across secondary periods did not affect individual capture probabilities.

Survey effort, expressed in km of transects surveyed during each secondary period, was included in the modelling processes as a covariate to test the effect on capture probabilities. All models with survey effort as a covariate performed very poorly for both humpback and snubfin dolphins (ΔAICc > 1000). This may be interpreted to indicate relatively small effects on the probability of capture from variation in effort at levels above the minimum achieved here. For both species, models including heterogeneity in capture probabilities also performed very poorly (ΔAICc > 20). Furthermore, no significant correlation (R) was found between the km of transect surveyed, number of groups sighted or number of marked individuals captured (sd: $R_{km\text{-}groups} = 0.09$, *p*-value = 0.73; $R_{km\text{-}p} = 0.26$, p-value = 0.34; hd: $R_{km\text{-}groups} = -0.03$, *p*-value = 0.91; $R_{km\text{-}p} = -0.11$, p-value = 0.67).

The ERMP survey area is the largest region (1147 km^2) ever surveyed in Australia in a single dolphin monitoring project. The majority of studies completed to date covered areas of less than 400 km^2 (Parra and Cagnazzi 2016). The Darwin Harbour Coastal Dolphin Monitoring Program (DHCMP) in The Northern Territory is the only exception, encompassing an area of about 1060 km^2 divided into three geographically separated small bays (Bynoe Harbour, Darwin Harbour and Shoal Bay) (Brooks et al. 2017). The survey design applied in the DHCMP project was however substantially less intensive than that applied in this study. Both the DHCMP and ERMP dolphin project followed sampling procedures based on robust design model structure. However, the DHCMP applied a Multistate Closed Robust Design Model, with each state being one of three bays, versus the CRDM used for the ERMP dolphin project. A multistate model with discrete geographic areas as states could not be applied in this study because the ERMP survey area is a single region with dolphins moving across the area within a single day.

The effort planned for this study was very intensive relative to typical dolphin surveys with an estimated coverage fraction of about 45% of the survey area in each secondary sample. The incompletion of some planned transects due to weather or tides is not expected to reduce the precision of the estimates to unacceptably low levels. Indeed, for snubfin dolphins (Port Alma), the RSE (CV) of the 3 annual estimates was below 0.20 with a mean of 0.15, and for humpback dolphins (all sites and overall), the RSE (CV) ranged between 0.05 and 0.21 with a mean of 0.09. These RSE values represent very high levels of precision that, with one exception (Port Alma in the third year), exceeded the recommended target of RSE ≤ 0.20. On average, this target was exceeded by some margin. Overall, the study has achieved levels of precision not often obtained in studies of these species. The survey design used in this study was considered appropriate to obtain robust and

reliable estimates of abundance and movement patterns of humpback and snubfin dolphins in the ERMP survey area.

2.4.2 Overview

The present study has provided the first population estimates of two coastal dolphin species for the entire ERMP survey area following the completion of the WBDDP in 2013. Overall, the study design provided reliable and precise estimates for humpback dolphins, while less precise abundance estimates were obtained for snubfin dolphins. Population estimates derived here indicate that ~ 110–140 adult snubfin plus about 17% of juveniles and calves, and 140–162 adult humpback dolphins plus about 36% of juvenile and calves used the ERMP survey area and Keppel Sands between May and September from 2014 to 2016. Indo-pacific bottlenose dolphins occurred only sporadically within the ERMP survey area and therefore data could not be analysed for population estimates.

Results from this study were compared with those obtained in the DHCMP project. The number of humpback (n = 159) and snubfin dolphins (n = 80) captured in five years in the Darwin region was substantially lower compared to the number of dolphins captured in three years in the ERMP survey area (humpback = 181 and snubfin = 127). Total population estimates of humpback and snubfin dolphins for the Darwin region (humpback dolphins ~ 90; snubfin dolphins 19 to 70) were also lower than those reported for the ERMP survey area. However, abundance estimates from the two projects are not directly comparable, since they were obtained for primary periods of different length, about one month for the DHCMP and five months for the ERMP. Finally, abundance estimates for Darwin Harbour region took into account the observed high permanent immigration and emigration to and from an unknown area (movement between bays in Darwin Harbour region was observed, but it was relatively limited). In contrast, both mark-recapture and genetic evidence suggested that the large majority of humpback and snubfin dolphins in the ERMP survey area were permanent residents and therefore available to be captured all year round. In both projects, humpback dolphins showed higher capture probabilities which resulted in more precise abundance estimates than for snubfin dolphins.

2.4.3 Abundance estimates for Australian snubfin dolphins

Abundance estimates for snubfin dolphins in this study have relatively large standard errors and confidence intervals and showed substantial variation across different models, indicating some level of uncertainty in the results. Average capture probabilities (p_1 ~ 0.13, p_2 ~ 0.18, p_3 ~ 0.12) were below the value selected (p = 0.2) as a target for precision, and in one secondary period in each

primary period, capture probabilities were below ($p < 0.08$), the minimum value of 0.1 required to obtain reliable abundance estimates (White et al. 1982). Nevertheless, the RSE of the estimates remained below the upper criterion for study precision (RSE < 0.2) suggested in Brooks et al. (2014). Specifically, the abundance estimate of 143 marked snubfin dolphins in the second primary period appeared to be inflated from a large influx of newly marked individuals never captured again (n = 35).

Average estimates of apparent annual survival were low ($\phi \sim 0.68$) compared to other studies ($\phi >$ 0.80) (Brown et al. 2014a, Brooks et al. 2017). The low apparent survival and capture probabilities under standardised survey effort and sighting condition could be explained with the movements of marked animals to and from the sampling area, known to encompass only about 50% of the whole range of the local resident population which extends as far as Corio Bay at the northern end of Keppel Bay (Cagnazzi et al. 2013c). Temporary shifts in habitat use, in the absence of external disturbance, have been commonly associated with prey distribution. Snubfin dolphin ranging patterns in Keppel Bay may reflect the temporal and spatial dynamics of their prey, which varies on a daily basis with the tidal cycle as well as year to year with differing dry or wet periods. When prey concentrates primarily outside the survey area, the majority of the dolphins may not be available for capture. Similarly, periods of exceptional productivity within the survey area may attract dolphins from a larger area resulting in higher capture probabilities and estimates. The presence of transient individuals and permanent emigrants (at least for a time longer than the length of the study) as suggested by the high number of newly caught individuals during the second primary period, can also result in lower apparent survival.

Genetic analyses conducted on biopsy samples collected from the only two known resident populations along the Queensland coast (Whitsundays to Port Alma ~ 450km apart) showed significant population structure at both mitochondrial and nuclear markers (Chapter 3). Accordingly, with the genetic evidence, dedicated surveys to the north of Port Alma to the Whitsundays resulted in very few and occasional sightings. Overall, based on the available information, it seems evident that Port Alma represents the only core habitat for snubfin dolphins south of the Whitsundays.

Port Alma supports a resident population ranging between approximately 100 and 163 individuals making this one of the largest populations of snubfin dolphins studied in Australia and comparable only to Roebuck Bay, Western Australia ($\widehat{N}_{total} \sim 130$) (Brown et al. 2016b). Abundance estimates presented in this study were similar (2014 and 2016) or higher (2015) than those recorded in the same general area between 2007 and 2010 ($\widehat{N}_{total} = 105$, 95%CI = 100–110 Cagnazzi et al. (2013c)).

2.4.4 Abundance estimates for humpback dolphins

Australian humpback dolphins were distributed over the whole area and found in most parts of the available habitats including the Narrows. Movements between sub-areas were observed, but were largely confined to movements between Port Curtis and Rodds Bay. However, for the first time we documented the movement of a single humpback dolphin through the Ramsey Crossing which suggests that The Narrows may be an important corridor to maintain connectivity between humpback dolphins in Port Alma and Port Curtis.

Overall, there was no evidence for temporary emigration longer than three months (the length of a primary period), but may occur for shorter periods. Based on previous knowledge of dolphin movements in the region, the large majority of the area used by the local populations has now been surveyed. Movements in and out the borders of the ERMP survey area (e.g. to Corio Bay) have been recorded to occur on a daily basis, and the majority of the dolphins (n = 77) observed between 2014 and 2016 were observed consistently during surveys prior to this study, which indicate a high proportion of resident individuals. Analysis of genetic data also confirmed that the large majority of humpback dolphins in the ERMP survey area (~ 93%) are resident and that migration to and from nearby populations, from Whitsundays to the north and Great Sandy Strait to the south, is extremely low (Chapter 3).

The number of humpback dolphins present in the entire ERMP survey area and Keppel Sands during each primary period varied from ~ 162 in 2014 and 2015 to 140 in 2016. Average capture probabilities for each primary season were > 0.23±0.02 which exceeds the minimum target of 0.2 established in simulations as the best trade-off between obtaining reliable precise results while minimising survey costs. The RSE for all estimates were < 0.1 indicating that the sampling regime provided acceptable precision for estimates of the abundance of humpback dolphins.

A multistate mark-recapture model could not be applied in this study because movement between sites were mostly observed within the secondary period and often within the same day, breaking the assumption of geographic closure required in multistate models. Therefore, the ERMP survey area was divided into three major areas: Port Alma/Keppel Sands, Port Curtis and Rodds Bay using available knowledge of dolphin movement together with well-defined existing physical boundaries. Population estimates were provided for Port Alma/Keppel Sands, Port Curtis plus Rodds Bay, and Port Curtis alone. No estimates were provided for Rodds Bay alone due to the large number of surveys without any captures.

The number of humpback dolphins using Port Alma and Keppel Sands varied from 68 in 2014 to ~ 36 in 2015 and 2016. The RSE of these estimates varied between 0.16 and 0.09 in 2014 and 2015 to

0.21 in 2016, which is above the target for precision set for this study. Capture probabilities were lower (~ 0.16) than those estimated from the entire data set but above the minimum value of 0.1 required to obtain reliable abundance estimates (White et al. 1982). Survival probability was very low 0.44 (SE = ±0.08) , which could be explained only partially with the movements of marked animals to and from the sampling area known to encompass about 50% of the whole range of the local population, which extended as far as north as Corio Bay, about 40 km north of Keppel Sands. The presence of transient individuals can affect apparent survival also, but the majority of individuals captured between 2014 and 2016 were well known long-term residents and only four new individuals were added to the database between 2015 and 2016. Despite the higher RSE of the 2016 abundance estimates, the decline in the number of dolphins using Port Alma from 2014 to 2015 and 2016 was significant (not overlapping 95%CI). The causes of this decline are unknown, though possibly explained by a temporary shift in the distribution, or permanent emigration, and possibly some loss from mortality. Integration of data collected in this study with those collected by Dr Cagnazzi before 2014 will allow clarification on whether this decline is 1) permanent, 2) only a temporal shift in habitat use due to poor water quality affecting this region especially during floods periods, or 3) represents a permanent decline in the number of humpback dolphins using this region. In contrast, there was no evidence for a significant trend, either positive or negative, in the number of humpback dolphins using Port Curtis and Rodds Bay. The number of humpback dolphins that used this area during each primary period varied from 101 in 2014 to 124 in 2015 and back to 108 in 2016. The increase in population recorded in 2015 could be explained by an influx of few transient groups, and a total of 24 new individuals were added to the catalogue. None of these individuals was sighted during surveys conducted before 2014. Overall capture probabilities were high $p = 0.23$ (SE = ±0.02) resulting in highly precise estimates with RSE < 0.1. Apparent survival probabilities were also high and suggested that about 82% (SE = ±0.05) of the population was in either Port Curtis or Rodds Bay and available for capture in any primary season.

About 75% of the humpback dolphin population uses the Port Curtis area alone; total population estimates for this region were 76 dolphins in 2014, 85 in 2015 and 68 in 2015. Capture probabilities for each secondary period were mostly higher than the target value of 0.2 and the RSE of the abundance estimates were below 0.15, and therefore were within acceptable target for precision. The availability of a long-term dataset allows us to confidently assign the sex to a large proportion of individuals in the population, enabling the effect of sex to be tested in mark-recapture models. Models with sex effect were strongly supported in the Port Curtis dataset, and these indicated that females ($p = 0.68$, SE = 0.17) were more likely to be in the area and available for capture than males ($p = 0.24$, SE = 36). This may indicate some variation in habitat use by different sexes, with

males potentially ranging over larger areas than females to maximize mating opportunities with multiple females (Sprogis et al. 2016).

The estimates provided in this study cannot be directly compared with population estimates of humpback dolphins from the same general area between 2007–2011 since they are based on different models (Cagnazzi 2013). However, we can confidently assert that in 2014–2016 the number of humpback dolphins using Port Curtis returned to values similar to those recorded in 2007–2010 (average 2007–2010: $\widehat{N}_{total}$ = 80, SE = 4.9, 95%CI = 71–90) before a step decline recorded in 2011 ($\widehat{N}_{total} = 45$, SE = 7.7, 95%CI 30–61) following the flooding and the start of WBDDP (Cagnazzi 2011). In contrast, the number of humpback dolphins using the Keppel Bay region (including Port Alma) between 2007 and 2010 (average 2007–2010: $\widehat{N}_{total}$ = 114, SE = 6.55, 95%CI = 102–127) was substantially higher than those recorded in Port Alma between 2014 and 2016. However, Keppel Bay is a much larger area than Port Alma, therefore, for a better comparison, it is necessary to limit mark-recapture analysis to data collected from 2007 to 2011 in Port Alma only.

3 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 wild *Sousa chinensis* and *Orcaella heinsohni*, and (b) analysis of tissues collected opportunistically from the carcasses of these species from this region.

3.1 Introduction

Coastal ecosystems in Australia, and indeed around the world, face unprecedented threats associated with urbanisation, agricultural activities, and the development of port and shipping infrastructure (Grech et al. 2013). Destruction and degradation of coastal marine ecosystems through the continued intensification of human activities in coastal areas (Davidson et al. 2012, Dulvy et al. 2014) are the primary causes of declines in global and Australian biodiversity (Lotze et al. 2006, Worm et al. 2006, Halpern et al. 2008, Woinarksi et al. 2014). Populations of apex predators inhabiting coastal waters, such as humpback and snubfin dolphins are among the most threatened (Parra and Cagnazzi 2016, Hawkins et al. 2017).

The long-term survival of animal populations is regulated by demographic, environmental, and genetic factors (Shaffer 1981, Nunney and Campbell 1993). Genetic diversity has important consequences on both individual and population fitness, as well as population resilience and persistence, and to the ability of individuals and populations to adapt to sudden environmental changes (Hughes et al. 2008). There is usually a negative correlation between genetic diversity and extinction risk, which is greater in small fragmented populations. Habitat degradation and destruction typically leads to habitat and population fragmentation. In species with high levels of natal philopatry (i.e. where animals return to their birthplace to breed), this can result in further population fragmentation and discontinuities in gene flow (transfer of genetic material via mating opportunities) among populations that are potentially already fragmented or genetically isolated. In the long-term, this will lead to further sub-population differentiation and affected populations may face the risk of loss of genetic diversity by elevated rates of inbreeding and genetic drift (Furlan et al. 2012).

All available evidence suggests that, at the present time, humpback and snubfin dolphins exist as metapopulations, characterised by small populations with low migration and gene flow among them (Cagnazzi 2010, Brown et al. 2014b, Parra and Cagnazzi 2016). Low genetic diversity is

particularly concerning for species with a metapopulation structure, as they are more vulnerable to genetic stochasticity, such as inbreeding (increased probability of breeding with related individuals) and genetic bottleneck (marked reduction in genetic diversity followed by the survival and expansion of a small random sample of the original population) which may result in further loss of genetic diversity (Hamner et al. 2012). Loss of genetic variation affects population viability and increases the risk of extinction via factors such as susceptibility to disease and decline in reproductive fitness associated with increased homozygosity, inbreeding depression and loss of effective population size (Hanski 1998, Spielman et al. 2004, Frankham 2005, Weeks et al. 2016). Effective population size (N_e) is an important parameter in conservation genetics that represents the number of effective breeding individuals in the parental generation, and determines the extent of loss in genetic diversity in the subsequent generation.

In coastal regions adjacent to the Great Barrier Reef Marine Park, the human population has been growing at rates (40% increase from 2009) faster than the Australian average (Great Barrier Reef Marine Park Authority 2009). The ERMP survey area is one of the regions undergoing major coastal development along the Queensland coast and the cumulative impact of the development on marine coastal ecosystems has raised concerns for the long-term survival of Australia's inshore dolphins in this region (Parra et al. 2006a, Cagnazzi et al. 2013c, Meager and Limpus 2014, Woinarksi et al. 2014). To ensure the conservation of humpback and snubfin dolphins in the ERMP survey area, it is critically important to understand how genetic diversity is partitioned spatially within and among nearby populations, (i.e. to determine population genetic structure). Descriptions of population genetic structure enable inferences to be made about the levels and patterns of dispersal among populations, potential for differentiation among populations, and evolutionary history of populations (Frankham 2005).

In this chapter, results of epidermal/blubber biopsy samples collected from the ERMP survey area were combined with those collected from nearby populations (Cagnazzi 2011) to provide insights into the connectivity and demographic history of humpback and snubfin dolphins residing in the ERMP survey area. More specifically this data will improve scientific understanding of the patterns of genetic diversity, population structure, gene flow, migration rate (m), genetic bottleneck and effective population size of humpback and snubfin dolphins in the ERMP survey area with important ramifications for their conservation and management.

3.2 Material and methods

3.2.1 Biopsy sampling protocol

Biopsy samples from 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 a safe, cost-effective, commonly used method of obtaining skin samples from free-ranging dolphins and was co-developed by the co-investigator Dr. Michael Krützen (Krützen et al. 2002). Sampling is undertaken with minimal risk and disturbance to the dolphins because tissue samples are collected remotely through the use of darts, and animals do not require to be captured and/or handled (Krützen et al. 2002). While in the field, all samples were stored in liquid nitrogen, and then transferred to a -80°C freezer at SCU.

All biopsy samples were collected during boat surveys conducted between secondary periods. The aim of these trips 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 surveys were conducted in rotation throughout the entire ERMP survey area and Keppel Sands.

After a group of dolphins was sighted, the research team 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 samples were collected from dependent calves. A full biopsy sample plug (Figure 10) is about 5 mm^2 in size and it is composed by an epidermis and a blubber layer. Genetic analyses were run on both layers with preference for the epidermis, which includes best quality DNA. Figure 10 also shows which section of the biopsy samples was used for toxicological and stable isotope analyses described in Chapter 4 and 5 respectively.

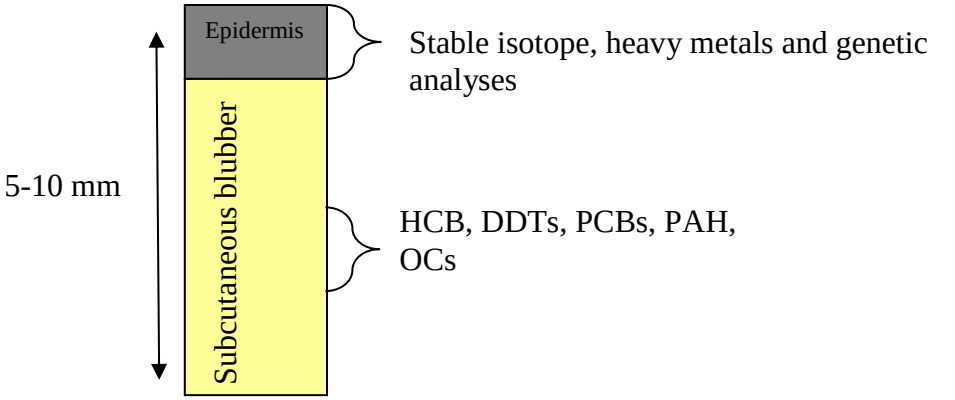

Figure 10 Representation of a biopsy sample taken from snubfin and humpback dolphins showing different sections used in various analyses.

3.2.2 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). The sex of the animals biopsied was determined by amplification of the ZFX and SRY genes (Gilson et al. 1998) through the polymerase chain reaction (PCR). 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 30 sec, 58^{o}C for 30 sec, 72^{o}C for 60 sec and 72^{o}C for 10 sec.

3.2.3 Mitochondrial DNA screening and sequencing

A DNA fragment of approximately 400 base pairs was amplified using PCR. PCR set up consisted of 1µl of diluted template, 0.4 µl of Dlp-1.5 (5'-TCACCCAAAGCTGRARTTCTA-3') and Dlp-5 (5CCATCGWGATGTCTTATTTAAGRGGAA-3') at 10 µM, 0.4 µl of dNTPs 10 mM, 0.25 µl of $MgCl_2$ 25 mM, 2 µl of buffer, 0.05 µl of Taq polymerase and 15.5 µl of doubled distilled water for a final reaction volume of 20 µl (Krützen et al. 2004). PCR conditions consisted of an initial denaturation at 94°C/4 min, followed first by a touchdown cycle with annealing temperature decreasing of 1° per cycle from 63°C to 55°C (1 min) repeated for nine cycles and final extension at 72°C for 1 min. A cycle of 94°C (30 sec), 52°C (30 sec) and 72°C (1 min) was repeated 29 times and completed with a final extension of 10 min at 72°C. PCR products were tested by gel electrophoresis. Successfully amplified products were cleaned using a QIA quick purification kit (Quiagen). One µl of purified product was amplified using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) following the manufacturer's instruction. PCRs were run on Verity 96-Well Fast Thermal Cycler (Applied Biosystems). Sequencing fragments were detected on

an ABI PRISM 3730 DNA Analyser (Thermofisher). Sequences were edited using SEQUENCING ANALYSIS Software, version 5.2 (Applied Biosystems).

3.2.4 Microsatellite genotyping

Biopsy samples were genotyped at 30 microsatellite loci: 10 dinucleotide markers: F10, EV37 (Valsecchi and 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 µl 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 3 min at 95°C. Samples were run on an ABI PRISM 3730 DNA analyser and analysed with GeneMapper software version 4.0 (Applied Biosystems).
Four internal control samples were run to compare sizes across trays, and a subset of randomly selected samples (5-10 %) were repeated for all loci. Alleles were called using the program GENEMAPPER v.3.7 (Applied Biosystems).

3.2.5 Genetic diversity within population

For microsatellite data, the program MICROCHECKER was used to investigate the presence of genotyping errors or scoring errors, null alleles and large allele dropout (Van Oosterhout et al. 2004a). The program GENEPOP v.3.4 (Raymond and Rousset 1995) was used to test for deviations from Hardy-Weinberg equilibrium (HWE) for all polymorphic loci and each population and to test for linkage disequilibrium between all pairs of loci and for each population (1,000 dememorisation iterations, 1,000 batches, 10,000 iterations per batch). The program GenAlEx 6.5 (Peakall and Smouse 2012) was used to calculate the mean number of alleles (NA), observed (H_o) and expected (H_e) heterozygosity and inbreeding coefficient (F_{IS}). Allelic richness was calculated using FSTAT v.2.9.3 (Goudet 1995). Bonferroni corrections were applied when required to adjust for multiple comparisons. The program GenAlEx 6.5 was also used to identify potential replicate samples by

calculating the probability of identity (P_{ID}), or chance that a pair of randomly selected individuals will have matching genotypes.

Alignment of mtDNA sequences was carried out using SeqMan software (DNAstar). Arlequin 3.5.1.3 (Excoffier et al. 2005), was used to summarise genetic diversity as the proportion of polymorphic sites, haplotype diversity (*h*) and nucleotide diversity (π).

3.2.6 Genetic diversity and population differentiation

Genetic differentiation among sampling locations and populations, i.e. those sampling locations that were later combined into a single putative population based on the STRUCTURE output (see below) was estimated with three different indexes F_{ST}, Jost' D_{EST} and Shannon's mutual information index ($^{S}H_{UA}$) using GenAlEx v.6.5 (Peakall and Smouse 2012). Significant differences among sampling locations and putative populations using the above indexes was tested based on 9,999 permutations and all multiple comparisons were Bonferroni corrected. Arlequin 3.5.1.3 was used to run an AMOVA on mtDNA data.

The program STRUCTURE 2.3.4 (Pritchard et al. 2000) was used to evaluate the most likely number of putative populations (K) by assigning individuals to clusters without previous information of population numbers and limits. Analyses in STRUCTURE v. 2.3.4 were run using the admixture model with correlated allele frequencies, with information on sampling location to improve clustering without spuriously inferring structure if absent (Hubisz et al. 2009). Five independent runs were performed for up to six possible K, with 1,000,000 Markov chain Monte Carlo (MCMC) steps and a burn in of 100,000. The most likely number of genetically homogeneous clusters was determined using the standard method (Pritchard et al. 2000) based on the posterior probability of the data (LnP(D)) and Evanno method (Evanno et al. 2005). Both methods were implemented in STRUCTURE HARVESTER (Earl 2012). STRUCTURE outputs were processed with CLUMPAK (Kopelman et al. 2015) which assigns groups of runs to a common clustering pattern and for graphical representation of results.

3.2.7 Migration rates and contemporary effective population sizes and bottleneck

Estimates of recent migration rates and the magnitude and direction of contemporary gene flow between sampling locations and populations were determined using BAYESASS 3.0 (Wilson and Rannala 2003) a molecular assignment program that relies on a non-equilibrium Bayesian approach method through MCMC techniques. Five independent runs with different random number seeds were run to check consistency of the results between the runs. Each run was completed with a

default setting of 10,000,000 iterations, a burn-in period of 1,000,000, and sampling frequency of 2,000.

The bias-corrected version of the linkage disequilibrium method (Waples 2006, Waples and Do 2010, Peel et al. 2013), as implemented in the program NeEstimator V2 (Do et al. 2014), was used to estimate contemporary genetic effective population size for each sampling location and populations identified by STRUCTURE analysis. The linkage disequilibrium method is suited to microsatellite data and has proven to be quite powerful in estimating effective population size with use of 10–20 microsatellite loci and samples of at least 25–50 individuals for species with overlapping generations, and small sample and population sizes (< 500) (Waples and Do 2010). Low frequency alleles (critical *p*-value < 0.05) were excluded from analysis.

Whether humpback or snubfin dolphins had undergone a recently genetic bottleneck in the ERMP survey area was tested using the software BOTTLENECK (v1.2.02) (Piry et al. 1999) with 10,000 iterations, and the Wilcoxon sign rank tests was used to assess significance. BOTTLENECK v1.2.02 provides results for three models of the generation of new alleles; the stepwise mutation model (SMM), the infinite allele model (IAM) and the two-phased model of mutation (TPM). Among these, the TPM model has been shown to be more appropriate for microsatellite DNA (Di Rienzo et al. 1994).

3.3 Results

3.3.1 Sample collection

During the ERMP project a total of 33 humpback dolphin and 36 snubfin dolphin biopsy samples were collected between 2014 and 2016, all from free ranging dolphins (Figure 11). DNA was successfully extracted from 64 samples (humpback dolphins = 29; snubfin dolphins = 35). The DNA concentrations in the remaining five samples were less than 1 ng/µl and gel electrophoresis failed to detect any DNA (Table A.7). In addition to those samples, genetic data from 38 humpback and 21 snubfin dolphin samples collected in the ERMP survey area, 12 humpback and 21 snubfin dolphin samples from the Whitsunday and 30 humpback dolphin samples from the Great Sandy Strait (Figure 11) were also included in the analysis to investigate gene flow and migration rates across a wider geographic area. These samples were collected between 2008 and 2011 as part of Daniele Cagnazzi PhD thesis and have been used in a larger project aiming to assess the genetic population structure of these species in Queensland (Parra et al. unpublished data).

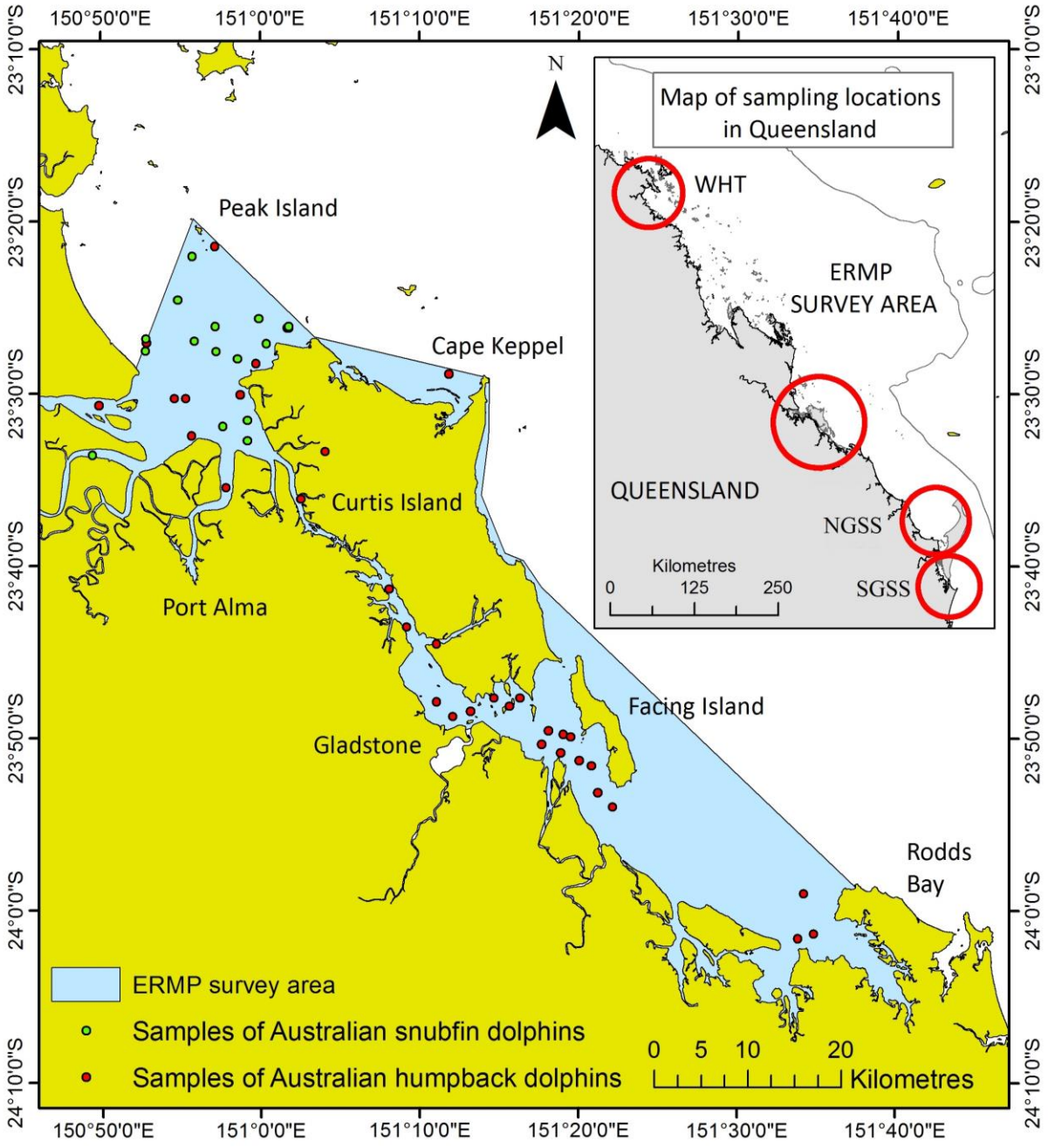

Figure 11 Map of humpback and snubfin dolphin biopsy samples collected in the ERMP survey area from 2014–2016 (left) and of additional sampling locations sampling included in the analysis (right). SGSS = Southern Great Sandy Strait, NGSS= Northern Great Sandy Strait, and WHT = Whitsundays.

3.3.2 Australian humpback dolphin genetic diversity and population differentiation

A total of five samples of humpback dolphins were replicated and used to assess error rate. Independent scoring of the same individual revealed an error rate of 0.29%. Of the 27 microsatellite loci amplified in humpback dolphin samples, 18 were polymorphic (more than one allele per locus). From the remaining polymorphic loci, evidence of null alleles was detected at loci EV37 and MK5,

significant deviation from HWE was found in Locus Tur4_141 and Locus Tur4_91 failed to amplify for about 28% of the samples. Thus, loci EV37, MK5, Tur4_141 and Tur4_91 were all removed from the analysis.

Using 14 polymorphic loci for each sample, the probability that two unrelated individuals shared a similar genotype was very low ($P_{ID} < 2.8 \cdot 10^{-4}$). Results from identity analysis using microsatellite polymorphic loci revealed the presence of four identical matching pairs and one from each pair was removed from the analysis. The final dataset included a total of 111 humpback dolphin samples; 69 from Port Alma (n = 38) and Port Curtis (n = 31), 12 from the Whitsundays and 30 from the Great Sandy Strait (Table 10). None of the remaining 14 loci showed the presence of null allele, significant deviation from HWE across all populations or significant linkage disequilibrium for any pair of loci after sequential Bonferroni correction (Table A.8 and A.9). Levels of microsatellite diversity were very low and similar across populations (Tables 10).

A fragment of about 400 base pairs of the mtDNA was amplified for 94 samples (Table 10). Sequence analysis of the control region revealed nine variable sites, defining seven unique haplotypes (Table 10). Only one haplotype occurred in all five sample sites, and this haplotype was also the most common (70 samples). Overall, nucleotide diversity was very low (Table 10) and no genetic differentiation was found between sampling location based on mtDNA ($\Phi_{ST} = 0.00$, *p*-value = 1.00).

Table 10 Measures of genetic variability based on 14 microsatellite loci of humpback dolphins for the five sampling locations. In the table, n = sample size; NA = mean number of alleles, AR = allelic richness, H_o = observed heterozygosity, H_e = expected heterozygosity, F_{IS} = inbreeding coefficient, nh = number of haplotypes. Values in parentheses are standard errors. WHT = Whitsundays, PC = Port Curtis, π = haplotype diversity. PA = Port Alma, NGSS = Northern Great Sandy Strait, and SGSS = Southern Great Sandy Strait.

	Microsatellite						**mtDNA**		
Sites	**n**	**NA**	**AR**	**H_o**	**H_e**	**F_{IS}**	**n**	**nh**	***π***
WHT	12	2.92(0.26)	2.43(0.20)	0.41(0.07)	0.39(0.03)	0.11(0.09)	8	5	0.011 (0.007)
PA	38	3.07(0.28)	2.27(0.22)	0.42(0.05)	0.42(0.05)	0.01(0.04)	33	3	0.006 (0.003)
PC	31	2.85(0.25)	1.82(0.20)	0.38(0.04)	0.38(0.04)	0.01(0.04)	23	5	0.007 (0.004)
NGSS	12	2.57(0.20)	2.33(0.20)	0.44(0.04)	0.39(0.03)	-0.11(0.05)	12	1	0
SGSS	18	2.07(0.12)	2.58(0.26)	0.31(0.06)	0.28(0.04)	-0.07(0.07)	18	1	0

The hierarchical AMOVA analysis based on microsatellite data values suggested that about 90% of the variance was explained by variation within sampling locations, while less than 10% explained variation between. Significant overall genetic differentiation was found among all sampling locations ($F_{ST} = 0.106$, *p*-value = 0.00; Jost' DEST = 0.041, *p*-value = 0.04; $^{S}H_{UA} = 0.160$, *p*-value =

0). Follow up pairwise comparisons based on F_{ST}, Jost' DEST and $^{S}H_{UA}$ values were all significant (Tables 11)

Table 11(a and b) Pairwise fixation index values among sampled locations of humpback dolphins based on 14 microsatellite loci (F_{ST}, Jost' $D_{EST\ and}$ $^{S}H_{UA}$). WHT = Whitsundays, PC = Port Curtis, PA = Port Alma, NGSS = Northern Great Sandy Strait and SGSS = Southern Great Sandy Strait.

a) F_{ST} are below diagonal and Jost' DEST above diagonal and *p*-values are in parentheses.

Sites	NGSS	PA	PC	SGSS	WHT
NGSS		0.010 (0.00)	0.076 (0.00)	0.051 (0.00)	0.0074 (0.00)
PA	0.119 (0.00)		0.020 (0.00)	0.112 (0.00)	0.091 (0.21)
PC	0.101 (0.00)	0.027 (0.00)		0.109 (0.00)	0.090 (0.00)
SGSS	0.088 (0.00)	0.155 (0.00)	0.165 (0.00)		0.170 (0.00)
WHT	0.083 (0.00)	0.101 (0.00)	0.104 (0.00)	0.218 (0.00)	

b) Shannon's mutual information index ($^{S}H_{UA}$) are below diagonal, *p*-values above diagonal.

Sites	NGSS	PA	PC	SGSS	WHT
NGSS		0.00	0.00	0.00	0.00
PA	0.055		0.00	0.00	0.00
PC	0.052	0.022		0.00	0.00
SGSS	0.046	0.069	0.066		0.00
WHT	0.080	0.055	0.051	0.109	

The Bayesian clustering analysis implemented in STRUCTURE reached a plateau in LnP(D) values at K = 4, whereas based on the Evanno method the model with K = 2 showed the highest log-likelihood value (Figure 12). The graphical representation of K = 2 populations (Figure 12) grouped with high probabilities all samples from Port Curtis and Port Alma (ERMP survey area), and the Whitsundays in a single cluster, and samples from Southern and Northern Great Sandy Strait in a second separate cluster. For K = 4 samples, all samples from the ERMP survey area were grouped in a distinct cluster from the Whitsundays and Southern and Northern Great Sandy Strait. All samples collected in the Whitsundays were assigned with high probability to the Whitsundays cluster; whereas few samples collected from Port Curtis showed some level of admixture with the Northern Great Sandy Strait cluster (Figure 13).

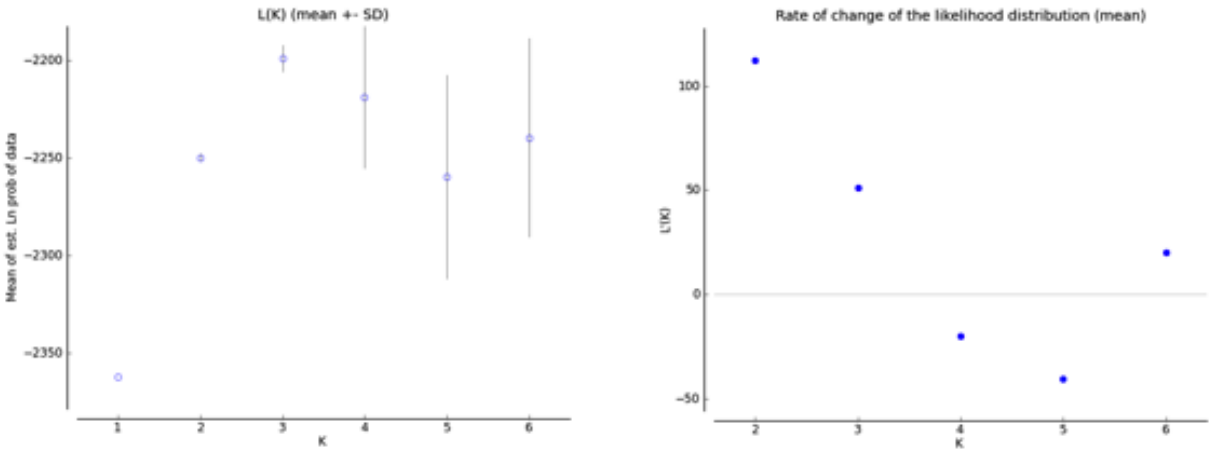

Figure 12 The most likely number of genetically homogeneous clusters based on Mean Ln posterior probability of the data (left) (Pritchard et al. 2000) and Evanno method (right) (Evanno et al. 2005) (averaged over 5 runs) estimated for the number of genetic clusters (K) ranging from 1 to 6. The most likely number of clusters was found to K = 3 for the standard method and K =2 respectively for the Evanno method.

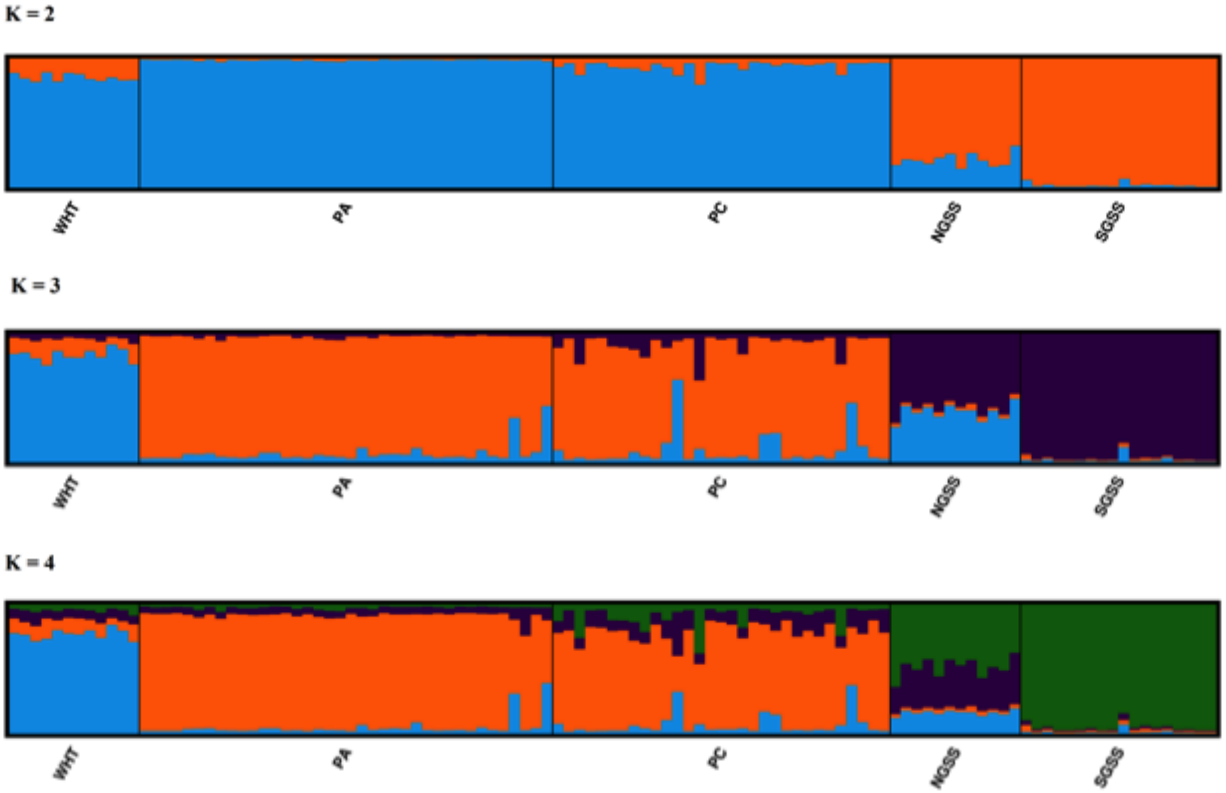

Figure 13 Structure plots showing estimated proportions of the coefficient of admixture of each individual's genome that originated from K = 2 to 4 populations of humpback dolphins. Individuals are grouped by sampling locations and sorted geographically from North (left) to South (right). WHT = Whitsundays, PA = Port Alma, PC = Port Curtis, NGSS = Northern Great Sandy Strait, SGSS = Southern Great Sandy Strait.

3.3.1 Australian humpback dolphin migration rates, effective population size and evidence of bottleneck

Contemporary migration rates revealed that the large majority of humpback dolphins in Port Curtis and Port Alma are resident (> 72%). A relatively high migration rate over one generation was estimated from Port Alma to Port Curtis (21%) but not vice versa (1.8%) (Table 12a). Estimates of migration rates into Port Curtis and Port Alma from other sampling locations were all extremely low (< 7.8%) (Table 12a). At the population level the large majority of individuals were classified as being non-migrants, and a moderate migration rate was estimated between the ERMP survey area and the Whitsundays (Table 12b).

Table 12 Matrix of inferred migration rates among sampled locations (a) and identified putative populations (b) of humpback dolphins calculated with the program BAYESASS 3.0. Values represent the fraction of individuals in the destination population that are migrants derived from the population of origin per generation. Values along the diagonal (bold) indicate the proportion of individuals per generation that are not migrants. Values in parentheses represent standard deviations. Sampling locations and putative populations are sorted geographically from North to South. WHT = Whitsundays, PA = Port Alma, PC = Port Curtis, NGSS = Northern Great Sandy Strait, SGSS = Southern Great Sandy Strait, ERMP = PC+ PA, GSS = NGSS + SGSS.

a) Migration rates among sampled locations

Origin Site	Destination site				
	WHT	**PA**	**PC**	**NGSS**	**SGSS**
WHT	**0.736 (0.055)**	0.006 (0.008)	0.006 (0.000)	0.001 (0.018)	0.004 (0.008)
PA	0.065 (0.051)	**0.963 (0.024)**	0.212 (0.048)	0.031 (0.031)	0.005 (0.009)
PC	0.075 (0.046)	0.018 (0.019)	**0.728 (0.038)**	0.016 (0.022)	0.005 (0.009)
NGSS	0.078 (0.065)	0.005 (0.007)	0.018 (0.014)	**0.701 (0.034)**	0.235 (0.049)
SGSS	0.045 (0.043)	0.005 (0.008)	0.034 (0.025)	0.005 (0.009)	**0.978 (0.020)**

b) Migration rates among putative populations identified in this study.

Origin sites	Destination sites		
	WHT	**ERMP**	**GSS**
WHT	**0.733 (0.048)**	0.005 (0.006)	0.006 (0.008)
ERMP	0.158 (0.061)	**0.979 (0.016)**	0.008 (0.011)
GSS	0.108 (0.006)	0.014 (0.014)	**0.985 (0.014)**

The effective population sizes for the Port Curtis and Port Alma populations were estimated to be 31.2 (95%CI = 16.8–7720) and 42.2 (95%CI = 14.9–infinite), respectively. For the Port Alma population, there were conflicting results rearding recent bottlenecks depending on the method used (IAM *p*-value = 0.02, TPM *p* -value = 0.13, SMM *p* -value = 0.33). There was no evidence for bottleneck in the Port Curtis population (IAM *p* -value = 0.06, TPM *p* -value = 0.26, SMM *p* -value = 0.55). The mode shift test did not detect any distortion of allele frequency and showed a normal "L" shaped distribution for both populations, which is a typical property of a population in

equilibrium (Figure 14). The effective population size for the entire ERMP survey area was estimated to be 58 (95%CI = 35.1–118.0), and no evidence of a bottleneck was recorded when samples from Port Alma and Port Curtis were grouped together.

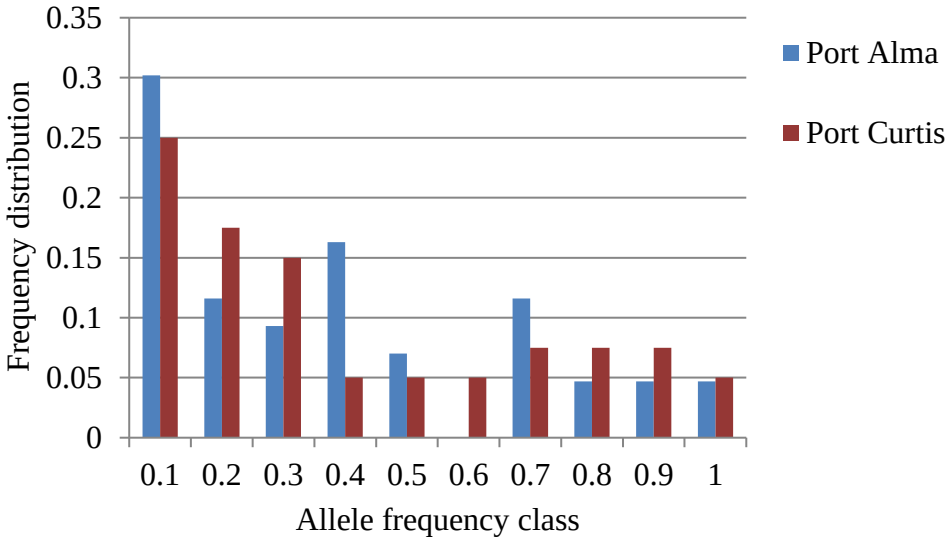

Figure 14 Allele frequency distribution visualising potential mode-shift distortion. The figures are based on 14 microsatellite loci for humpback dolphins in Port Alma (PA) and Port Curtis (PC).

3.3.2 Australian snubfin dolphin genetic diversity and population structure

Of the 27 microsatellite loci genotyped, 25 amplified, of which only nine were polymorphic. The quality control analysis based on five samples revealed no disagreement between initial and secondary scores. Even using only nine polymorphic loci the probability that two unrelated individuals share a similar genotype was low ($P_{ID} = 1.1 \cdot 10^{-5}$). Results from identity analysis identified four possible matches, one of which was excluded from the analysis. Additionally, three samples were removed because they failed to amplify for more than three loci. In the remaining dataset (70 samples and nine polymorphic loci), there was no evidence for null alleles, allele dropout or scoring error, significant departure from HWE or linkage disequilibrium (Table A.10 and A11). However, about 10% of the data were missing at loci TUR_142, Tur4_80 and TUR4_87. Levels of microsatellite diversity were low and similar for both populations (Table 13). The AMOVA analysis indicated that variation between putative populations based on F_{ST} (F_{ST} = 0.031, *p*-value = 0.001) $^{S}H_{UA}$ ($^{S}H_{UA}$ = 0.032, *p*-value = 0.001), and Jost' DEST values (Jost' DEST = 0.014, *p*-value = 0.102) were all significant.

Analysis of 421 base pairs of the mtDNA control region from 47 samples revealed seven unique haplotypes, of which five occurred in the Whitsundays and two in Port Alma. Nucleotide diversity in both sampling locations was low (Table 13). The most common haplotype was found in 76% of the samples from Port Alma and 57% from the Whitsundays, and was also the only haplotype

shared between the two populations. No genetic differentiation was found between the two sampling locations based on the mtDNA (Φ_{ST} = 0.00, *p*-value = 0.999).

The program STRUCTURE was used to test for population structure. Both the Mean Ln posterior probability of the data and the Evanno methods failed to clearly define the most likely number of populations (Figure 15). The graphic representation for K = 2 populations corresponding to the two sampling locations was presented in Figure 16. All samples collected from the Whitsundays were assigned with high probability to one distinct cluster, whereas all samples from Port Alma showed different levels of admixture with the Whitsundays cluster.

Table 13 Measures of genetic variability based on nine microsatellite loci of snubfin dolphins for the two sampling locations Whitsundays (WHT) and Port Alma (PA). In the table n = sample size; NA = mean number of alleles, AR = allelic richness, H_o = observed heterozygosity, H_e = expected heterozygosity, F_{IS} = inbreeding coefficient, nh = number of haplotypes, π = nucleotide diversity. Values in parentheses represent standard error.

Sites	**n**	**NA**	**AR**	**Ho**	**He**	**F_{IS}**	n	**nh**	**π**
WHT	21	3.67(0.41)	3.34(0.36)	0.44(0.06)	0.48(0.05)	0.07(0.05)	21	5	0.003(0.002)
PA	49	3.66(0.29)	3.52(0.35)	0.42(0.07)	0.44(0.07)	0.03(0.02)	26	2	0.003(0.002)

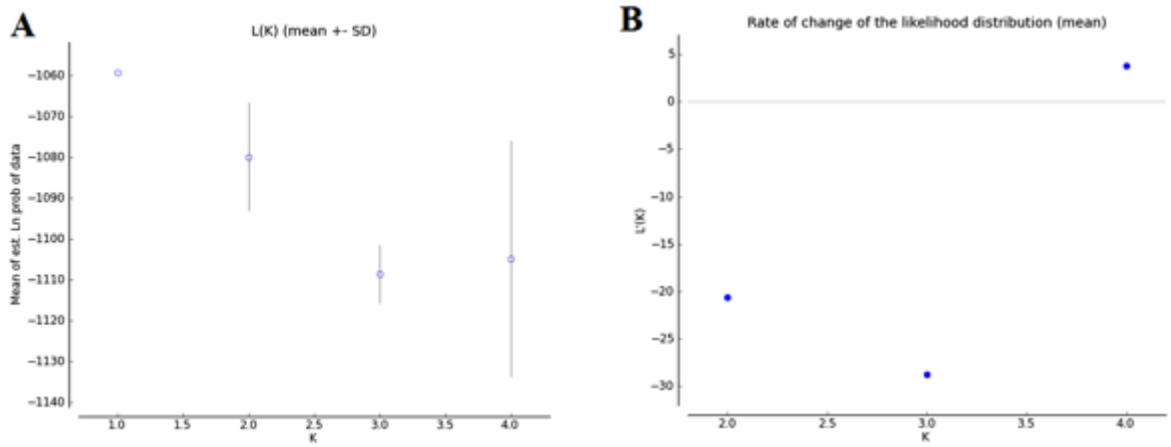

Figure 15 The most likely number of genetically homogeneous clusters based on mean Ln posterior probability of the data (A) (Pritchard et al. 2000) and Evanno method (B) (Evanno et al. 2005) (averaged over five runs) estimated for a number of genetic clusters (K) ranging from 1 to 4. WHT = Whitsundays and PA = Port Alma.

Figure 16 Structure plots showing estimated proportions of the coefficient of admixture of each individual's genome that originated from K = 2 populations. Individuals are grouped by sampling locations in the Whitsundays (WHT) and Port Alma (PA).

3.3.3 Australian snubfin migration rates effective population size and evidence of bottleneck

Analysis of contemporary migration rate indicated that the large majority of snubfin dolphins (~ 94%) living in Port Alma remained in the area (m = 0.94, SD = 0.03, 95%CI = 0.87–0.99). The proportion of resident individuals in the Whitsundays was also high but substantially lower than in Port Alma (m = 0.74, SD = 0.04, 95%CI = 0.68–0.85). A large proportion (~ 25%) of Port Alma snubfin dolphins appear to derive from the Whitsundays (m = 0.25, SD = 0.04, 95%CI = 0.14–0.31), whereas migration rates from Port Alma to the Whitsundays is low (m = 0.05, SD = 0.03, 95%CI = 0.01, 0.12). Estimates of effective population size for the Port Alma population were imprecise (N_e = 483, 95%CI = 48.5–infinite) due to low statistical power. There was no evidence for bottleneck in both populations (PA: TPM *p*-value = 0.35; WHT: TPM *p*-value = 0.58) and the mode shift test did not detect any distortion of allele frequency (Figure 17).

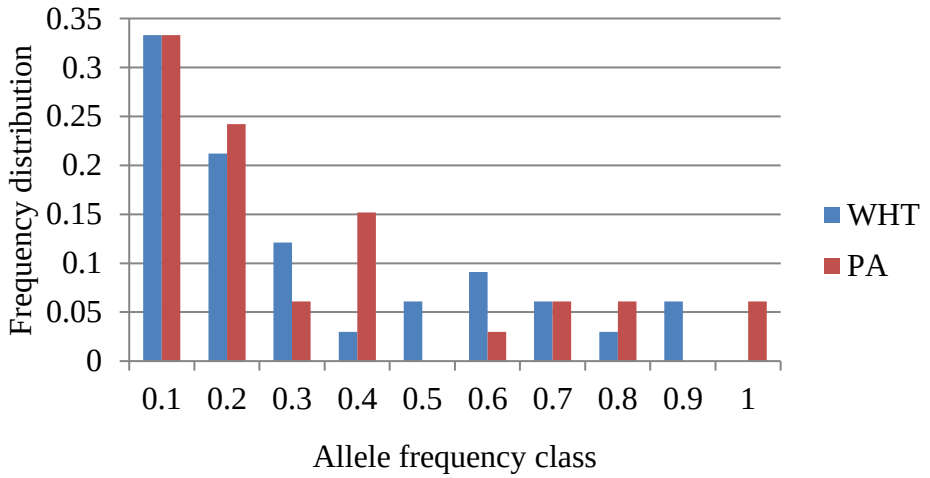

Figure 17 Allele frequency distribution visualising potential mode-shift distortion based on nine microsatellite loci for samples snubfin dolphins collected from the Whitsundays (WHT) and Port Alma (PA).

3.4 Conclusions

3.4.1 Genetic diversity

Remarkably low levels of genetic diversity were detected in nuclear DNA markers for both humpback and snubfin dolphins across all sampling locations. This low genetic variation falls within the range of values observed for cetaceans in small populations sizes (i.e. ~ 100 individuals) (Hamner et al. 2012). These findings are consistent with the current known abundance estimates for both species of less than 200 individuals for the entire ERMP survey area (Chapter 2). The ERMP survey area is home to the southernmost population of snubfin dolphins. Humpback dolphins are also close to southern end of their distribution, extending to Moreton Bay about 400 km south of the survey area. The decreasing genetic diversity observed along the coast (Parra et al, unpublished data) suggests that these populations might have originated via serial founder events, when northern populations expanded their range further south, incrementally loosing genetic variation. Local adaptation and natal philopatry can further result in the reduction of genetic diversity and the development of a metapopulation structure (Tezanos-Pinto et al. 2009), like that observed in this study.

The very low levels of genetic diversity may be a cause for concern. The importance of genetic variation relates to multiple aspects of population resilience and persistence, and is usually assumed to be critical for long-term fitness and adaptation (Frankham 2005, Willoughby et al. 2015).

3.4.2 Genetic population structure in Australian humpback dolphins

Overall, considerable genetic differentiation was detected in humpback dolphins among all sampling locations, suggesting the existence of discrete groups connected by limited gene flow. Despite the high level of genetic differentiation, STRUCTURE grouped the large majority of the samples collected in Port Curtis and Port Alma in the same cluster, with only one individual predominantly assigned to the Whitsunday cluster. The pattern of genetic structure observed between Port Curtis and Port Alma matched expectations based on the analysis of photo-identification data available between these two locations. Analysis of photo-identification data collected between 2006 and 2008 suggested the presence of two social communities connected by only a few focal individuals (Cagnazzi 2010). During boat-based surveys conducted as part of this current study, only five humpback dolphins were matched between Port Curtis and Port Alma, further confirming the movement of individuals between these two regions, albeit limited (section 2.3.2).

Estimates of contemporary emigration rates across sampling locations suggested that about 21% (95%CI = 0.11–0.29) of humpback dolphins in Port Curtis derived from Port Alma, whereas only 1.8% (95%CI = ~ 0–0.07) of the humpback dolphins in Port Alma derived from Port Curtis. These results suggest that Port Alma might act as a source population for humpback dolphins in the area. Estimates of migration rates also suggest that a larger proportion of humpback dolphins move away from Port Alma or Port Curtis in the direction of the Whitsundays (~ 6–7%) rather than from the Whitsundays to Port Alma or Port Curtis (~ 0–1%).

In summary, the overall population structure appears to be explained by the separation of humpback dolphins into three discrete management units (Moritz 1994, Palsboll et al. 2007) corresponding to: 1) ERMP survey area (Port Alma and Port Curtis), 2) Whitsundays and 3) Great Sandy Strait (Northern and Southern Great Sandy Strait). Significant genetic differentiation was found among all three putative populations. The ERMP survey area population showed a high proportion of non-migrants (~ 97%), with a moderate migration rate only in the direction of the Whitsundays (~ 15% over one generation corresponding to about 20 individuals over 20 years).

We acknowledge that distances between sampling locations were relatively large (ERMP to Great Sandy Strait ~180 km; ERMP to the Whitsundays ~ 400 km) and other populations are known to exist between the ERMP survey area and the Whitsundays. Port Clinton, located 100 km north of the ERMP survey area, is one of the areas that humpback dolphins are known to be resident north of the ERMP survey area. A photo-identification study conducted in Port Clinton from 2008–2010, resulted in no photographic matching with humpback dolphins identified from the ERMP survey area (D. Cagnazzi unpubl. data). Based on anecdotal information and unpublished data, the maximum observed distance travelled by an individual humpback dolphin in Australian waters is about 130 km and was recorded in the ERMP survey area (D. Cagnazzi unpubl. data).

The large geographic distance between populations and suitable habitats in Central and Southern Queensland are likely the key drivers of genetic differentiation documented in this study. Supporting this hypothesis is the pattern of isolation-by-distance detected from a larger study, which includes numerous sampling locations along the Queensland coast (Parra et al. in review). The distance between the ERMP survey area and the Great Sandy Strait (~ 180 km) is not substantially greater than between the ERMP survey area and Port Clinton (~ 100 km). Therefore, the collection of samples from Port Clinton would be fundamental to improve our understanding of the population structure and genetic boundaries of humpback dolphin in the ERMP survey area.

Based on the results of this study, humpback dolphins in the ERMP survey area should be considered as a separate management unit for conservation. However, it is important to note that the contemporary asymmetric gene flow found from Port Alma to Port Curtis suggests that Port Alma

might act as a source population for humpback dolphins in Port Curtis. This suggests that although the short-term survival of humpback dolphins in Port Alma and Port Curtis may not be directly dependent upon one another, the long-term survival of humpback dolphins in Port Curtis may be linked to the survival of humpback dolphins in Port Alma. Humpback dolphins in Port Alma appeared to have undergone a significant decline in abundance (section 2.3.4), thus long-term conservation actions should be directed toward the protection of dolphins in both localities, and the maintenance of a genetic corridor between them.

3.4.3 Genetic differentiation and population structure of Australian snubfin dolphins

Analyses of genetic diversity and population structure of snubfin dolphins was limited to Whitsundays and Port Alma. These are the only two locations south of Townsville known to support a resident population of snubfin dolphins. Dedicated surveys to assess the occurrence of snubfin dolphins between the Whitsundays and Port Alma were completed by Daniele Cagnazzi between 2006 and 2011, and new and ongoing surveys started in 2014. During these surveys, only two groups of snubfin dolphins were sighted between Port Alma and the Whitsundays, one in Shoalwater Bay and the other in Broadsound, both groups were composed of about 6–8 dolphins (D. Cagnazzi unpubl. data). Both groups were very elusive and the photographs were not suitable for matching purposes. The results of these surveys suggest that no other resident population of snubfin dolphin exists between Port Alma and the Whitsundays (Cagnazzi et al. 2013c). However, with consideration to few stranding data, occasional field observations and the low migration rate reported in this study, limited movements of snubfin dolphins seem to occur between Port Alma and the Whitsundays.

Significant population structure was estimated between the two sampling locations. Contemporary migration rates revealed that about 94% of the snubfin dolphins in Port Alma are resident. Of these, about 25% are derived from the Whitsundays, whereas less than 5% of the snubfin dolphins in the Whitsundays are derived from the Port Alma population. Therefore, the Whitsundays population might act as a source population for snubfin dolphins in Port Alma.

STRUCTURE assigned all samples collected from the Whitsundays with high certainty to the same cluster, whereas a high level of admixture was evident among several samples collected in Port Alma. Five individuals sampled in Port Alma were predominately assigned to the Whitsundays cluster suggesting that they were Whitsundays migrants, whereas all the other dolphins may be of migrant ancestry (Figure 16). Along with the genetic evidence, photo-identification data on snubfin dolphins in Port Alma have revealed that a large proportion of the marked individuals returned in the same area from year to year, but that occasional bursts of new individuals have entered the area

for shorter periods of time (see paragraph 3.3). Considering the available knowledge on snubfin dolphins along the Queensland coast, Port Alma may represent one of the few core habitats for snubfin dolphins in Central and Southern Queensland.

3.4.4 Effective population size and evidence of bottlenecks in humpback and snubfin dolphins

The effective population size provides an indicator of the number of individuals contributing genes to the next generation, and is thus considered a very important parameter to assess the conservation status and long-term viability of animal populations (Frankham 2005). By definition, effective population size is usually lower than the census size and, by definition, describes the rate of inbreeding accumulation and loss of genetic diversity (Robinson and Moyer 2013). It is generally accepted that effective population size should not fall below 50 to prevent inbreeding depression over five generations, and effective population size > 100 is required to limit loss in total fitness to < 10% (Frankham et al. 2014). A population with effective population size < 50 individuals and subject to population decline should be considered in a critical state (i.e. 50% probability of extinction within five years or two generations) (Mace and Lande 1991).

Our estimates of effective population size showed wide confidence of intervals, probably as a result of the lower number of polymorphic loci (humpback = 14, snubfin = 9) used in the analysis compared to what suggested for the linkage disequilibrium method (~ 20).

Effective population size estimates for humpback dolphins in Port Alma (effective population size = 31.2, 95%CI = 16.8–77.2) and Port Curtis (effective population size = 42.2, 95%CI = 14.9–infinite) are both below the critical limit of 50, with upper confidence intervals for Port Alma populations close to the critical limit. Estimates of effective population size for the entire ERMP survey area appeared to be lower (effective population size = 58, 95%CI = 35–118) compared to the sum of the separate estimates for Port Curtis and Port Alma. It must be noted that the Linkage disequilibrium models assume a closed and unstructured population (Waples 2006). However, in this analysis samples from two different subpopulations were included and the total effective population size may be biased low as result of admixture between sub-populations (Waples and England 2011). The effective population size for snubfin dolphins appeared to be highly overestimated and with little precision (effective population size = 483, 95%CI = 48.5–infinite), probably as a result of the limited number of polymorphic loci (n = 9) which resulted in very low statistical power. Therefore, the estimates of effective population size and bottleneck for snubfin dolphins cannot be considered reliable and more informative loci are needed to improve these results.

A major concern remains for the very low effective population size estimates for humpback dolphins in Port Alma, with the upper 95%CI being close to the critical limit of 50 individuals. In

addition, some evidence for a bottleneck was also detected in humpback dolphins from Port Alma. However, these results are somewhat ambiguous and the significance level varied depending on the mutation model considered. Furthermore, there was no evidence of a bottleneck for humpback dolphins in Port Curtis, or for the entire ERMP survey area.

The wide confidence intervals for effective population size and ambiguity in bottleneck evidence across different mutation models indicate considerable uncertainty in these results. Even though these results should be interpreted with caution, they provide a first insight into the connectivity and demographic history of humpback and snubfin dolphins in the ERMP survey area.

3.4.5 Limitations of the data and development of a new methodology to obtain more robust estimates.

The small sample size coupled with low genetic diversity observed for both species in standard microsatellite and mtDNA markers, resulted in some level of uncertainty in genetic structure and wide confidence intervals in most parameter estimates. The number of available samples within populations was below the recommended sample size of 25–30 suggested for genetic studies based on microsatellite allele frequencies (Hale et al. 2012).

While it is extremely difficult and time demanding to increase the number of biopsy samples, with the advancement in genomic technique it is possible to substantially increase the number of informative loci at a more reasonable cost. Population structure studies based on genomic markers such as single nucleotide polymorphisms have been successfully used to obtain precise estimates of demographic parameters in populations with less than 10 samples (Leslie and Morin 2016, Vollmer and Rosel 2017). We used DNA extract from two samples of humpback and snubfin dolphins to test and adapt a new method recently published by Kistler et al. (2016) that allows the detection and scoring of a large number of polymorphic loci, even in species with depauperate genetic diversity. In summary, from this preliminary test we obtained at least 3,000 polymorphic loci in each species compared to the 27 used in this study based on standard genetic techniques. As the number of genetic polymorphic markers increases the accuracy and precision in important population parameters, such as effective population size, migration rate and its directionality, admixture, and genealogical relationships between individuals (‘relatedness’) also dramatically increases without the need of increasing sample size. This high number of polymorphic loci will allow us to use coalesce analyses within Bayesian frameworks to estimate effective population size, divergence, and gene flow at very high precision using fewer samples (~ 10). Dependent on funding availability, this new method could be used to reanalyse some of the already collected biopsy samples together with new samples from remote area like Port Clinton (see paragraph 6.2).

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

4.1 Introduction

At the beginning of 2011 in the Port of Gladstone, in response to a dramatic increase in marine wildlife diseases and mortality (Stephen et al. 2013, Meager and Limpus 2014, Flint et al. 2015, Dennis et al. 2016), there were widespread public concerns about the health status of the local waterways. As a result, Fisheries Queensland instated a temporary closure on all fishing in an area centred on Gladstone Harbour during September 2011 while the Queensland Government investigated a condition affecting some locally caught fish. The causes of the observed outbreaks in marine diseases were never fully resolved but a causation link with the major environmental changes that affected Port Curtis in the summer of 2011 was suggested (Stephen et al. 2013).
In the same period, the carcasses of six humpback dolphins were found stranded in Port Curtis. However, the causes of death could not be determined. This is a substantially higher number of deaths compared to those generally recorded in Queensland from January 1996 to December 2012 (Meager et al. 2012, Meager and Limpus 2014). An indirect link between sudden and inexplicable mass mortality events of cetaceans and high level of contaminants has often been proposed (Kuehl and Haebler 1995, Jepson et al. 2005). More specifically, chronic exposure to environmental contaminants accumulated through the food chain can have immunosuppressive effects resulting in outbreaks of potentially deadly diseases such as morbillivirus (Aguilar and Borrell 1994, De Swart et al. 1996, Martineau et al. 2002, Jepson et al. 2005, Venn-Watson et al. 2015).
Recent surveys of pollutant concentrations in Great Barrier Reef habitats have confirmed that nearshore marine sediments contain a range of contaminants (Brodie et al. 2012), from both anthropogenic and natural sources, known to be potentially dangerous to dolphin health. Many pollutants are initially taken up by organisms at the bottom of the food chain and are found in increasing concentrations in the tissues and organs of animals in higher trophic levels. Dolphins as top predators can be exposed to persistent organic pollutants predominantly by the intake of food.

Since cetaceans do not have sweat and sebaceous glands, fur, or active blood-water exchange via gills and have low capacity for degradation of organochlorines (OCs) they can be regarded as closed systems in which contaminants accumulate practically without opposition (Tanabe et al. 1994). As a result, inshore dolphins living in coastal waters close to agricultural and industrial activity tend to accumulate high concentrations of anthropogenic contaminants such as pesticides, aromatic hydrocarbons and heavy metals, and this exposure may increase their risk of disease (Casalone et al. 2014, Desforges et al. 2017).

Till date, very few studies described contaminants levels in Australian marine mammals due to the limited number of carcasses recovered and the challenges in collecting samples. A range of organochlorine pollutants were found in the blubber of humpback dolphins, snubfin dolphins, bottlenose dolphins (*Tursiops* spp.) and short-beaked common dolphin (*Delphinus delphis*) from Queensland (Vetter et al. 2001, Cagnazzi et al. 2013a, Weijs et al. 2016). In particular PCBs in blubber of some of these animals were at near or above levels (∑PCBs = 17,000 ng/g lw) known to have adverse health effects in marine mammals (Kannan et al. 2000, Jepson et al. 2005) including impairment of immune function (De Swart et al. 1996, Kannan et al. 2000), increased neonatal mortality (∑PCBs = 11,000 ng/g lw) (Schwacke et al. 2002), decreased reproductive rates (Aguilar and Borrell 1994, Jepson et al. 2005) and associated carcinoma (∑PCBs = 77,000 ng/g lw) (Ylitalo et al. 2005). In fewer occasions DDTs were also found at levels above toxicological thresholds for reproductive toxicity (2,000–3,000 ng/gr ww) (Barron et al. 2003) and immunotoxic levels reported in harbour seals (De Swart et al. 1996).

Information on heavy metals in Australian marine mammal is more limited compared to OCs. High to moderate cadmium (Cd), mercury (Hg) and selenium (Se) were recorded in samples of inshore bottlenose dolphins (*Tursiops aduncus*), offshore bottlenose dolphin (*Tursiops truncatus*) and short-beaked common dolphin collected from carcasses stranded in South Australia. The levels of Cd, Hg and Se in samples from South Australia were higher than concentrations found in biopsy samples collected from dolphins in the Northern Hemisphere (Lavery et al. 2008). In the same study, higher concentrations of Cd, lead (Pb), Hg, Se, and zinc (Zn) were recorded in inshore dolphins (inshore bottlenose dolphins) compared to offshore species (offshore bottlenose dolphin and short beaked common dolphin) (Lavery et al. 2008). The only study conducted on humpback dolphins in Australia found the majority of essential elements analysed within the baseline data reported in Bryan et al. (2007) and Stavros et al. (2007).

In the ERMP survey area, the input of agricultural and urban-sourced pollutants has been identified as a major threat to the coastal water quality and a range of contaminants have been detected in the water, sediment and biota (Haynes and Michalek-Wagner 2000, Jones et al. 2005, Melville et al.

2009, Angel et al. 2010). In this area, anthropogenic pollutants reach the marine habitat from a variety of sources spread across the region. These include air and water emissions from agricultural and grazing activities, several industrial sources, inland coal mines, shipping and handling, coal stockpiles, power station corrosion products, leachate from landfill, urban development and sewage treatment. Potentially dangerous anthropogenic contaminants in snubfin and humpback dolphins living in Port Curtis and Port Alma was confirmed from the analysis of 24 biopsy samples collected in 2010–2011 (Cagnazzi et al. 2013a). In particular, the concentrations of PCBs were at levels near, or above, the toxicological thresholds associated with immune and reproductive toxicity or population declines in other marine mammals.

The aim of this present study was to further improve our understanding of the toxicological status of humpback and snubfin dolphins in the ERMP survey areas by conducting analyses of heavy metals and persistent organic pollutants in biopsy samples collected from free ranging dolphins.

4.2 Methods

4.2.1 Sample Collection

The biopsy samples used for the extraction of contaminants were collected following the sampling protocol described in paragraph 3.2.1. To investigate the concentrations of contaminants, the epidermis layer of the biopsy sample was carefully removed from the blubber layer by cutting as close to the interface between the two layers as possible using a methanol-rinsed stainless steel blade. The epidermis layer was used for the extraction of HCB, DDTs and PCBs, whereas the epidermis for the extraction of heavy metals.

4.2.2 Organochlorines

Determination of HCB, DDTs and PCBs was performed at the Environmental Sciences Department, University of Siena, according to the U.S. Environmental Protection Agency (EPA) 8081/8082 Method modified (Marsili and Focardi 1996). Samples of subcutaneous blubber (50 to 300 mg) were lyophilized in an Edwards freeze drier for two days and extracted with n-hexane (gas chromatography grade, Merck) in a Soxhlet apparatus. Whatman cellulose thimbles (i.d. 25 mm, e.d. 27 mm, length 100 mm) used for extraction of the samples were preheated for about 30 min to 110°C and pre-extracted for 9 h in a Soxhlet apparatus with n-hexane, in order to remove any organochlorine contamination. Each sample was spiked prior to extraction with 2,4,6-trichlorobiphenyl (International Union of Pure and Applied Chemistry (IUPAC) number 30 Ballschmiter and Zell (1980) as a surrogate compound. PCB30 was quantified and its recovery

calculated for each sample. After a 9 h extraction with n-hexane, the samples were purified with sulphuric acid to first obtain lipid sedimentation. The extract then underwent liquid chromatography on a column containing florisil that had been dried for 1 h in an oven at 110°C. This further purified the apolar phase of lipids that could not be saponified, such as steroids like cholesterol. Decachlorobiphenyl (DCBP - IUPAC number 209) was used as an internal standard, where it was added to each sample prior to the extraction and included in the calibration standard (a mixture of Aroclor 1260, HCB and pp'- and op'-DDT, DDD and DDE). High resolution capillary gas chromatography was performed with an Agilent 6890N and a 63Ni ECD and an SBP-5 bonded phase capillary column (30 m long, 0.2 mm i.d.). The carrier gas was nitrogen with a head pressure of 15.5 psi (splitting ratio 50/1). The scavenger gas was argon/methane (95/5) at 40 ml/min. Oven temperature was 100°C for the first 10 min, after which it was increased to 280°C at 5°C/min. The injector and detector temperatures were 200°C and 280°C respectively. The extracted organic material (EOM%) from freeze-dried samples was calculated in all samples. Capillary gas-chromatography revealed about 30 PCB 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 PCBs were quantified as the sum of all congeners. These congeners constituted 80% of the total peak area of PCBs in the biopsy. Total DDT was calculated as the sum of the isomers op'DDT, pp'DDT, op'DDD, pp'DDD, op'DDE and pp'DDE. The results were expressed in ng/g lipid weight (lw) unless differently specified. The detection limit was 0.1 ng/kg (ppt) for all the OCs analysed.

4.2.3 Heavy metals

A total of about 0.1 g of epidermis were cut off the biopsy samples and freeze-dried in a Labconco freeze dryer at 80°C, 0.045 mBar until no moisture remained. The sample was then ground with a mortar and pestle. Liver samples were homogenised in the vials using an Omni International 240-watt Tissue Master 125 laboratory homogeniser. Each dried, homogenised sample was weighed in an acid-cleaned Teflon digestion tube. In each tube, 5 ml of 70% analytical-grade HNO3 was added and the tubes were sealed. After pre-digestion for 30 min the tubes were placed in a closed, high-pressure microwave system (MARS5, CEM Corporation). Following digestion, tubes were left to cool in a fume hood for approximately 30 min. Material from each digestion tube was made up to 25 ml with Milli-Q ultra-pure water. Diluted material was stored in sealed and labelled polypropylene sample vials at 4^{o}C until analysis. Analyses of metals were completed at the National Association of Testing Authorities accredited Environmental Analysis Laboratory at SCU. Analytes measured in the sample digests included mercury (Hg), arsenic (As), cadmium (Cd), copper (Cu),

iron (Fe), selenium (Se), zinc (Zn), chromium (Cr), silver (Ag), manganese (Mn), nickel (Ni) and 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 (E.A.L. 2013, Gilbert et al. 2015). ICP-MS detection limits (limits of reporting) for the metals analysed were Hg < 0.0002 mg/l, As < 0.0007 mg/l, Cd < 0.00004 mg/l, Cu < 0.0002 mg/l, Fe < 0.005 mg/l, Se < 0.00025 mg/l, Zn < 0.003 mg/l, Ag < 0.1 mg/l (E.A.L. 2013). Further quality control measures included the use of duplicate blanks and internationally certified reference material in each analytical run of 20 samples. Concentrations of heavy metals were expressed in mg/kg wet weight (ww).

4.2.4 Data analysis

Based on photo-identification data, humpback dolphins in the ERMP survey area were known to be divided into two social communities with distinct home ranges corresponding to Port Alma and Port Curtis (Cagnazzi 2011). Port Alma and the surrounding catchment are primarily used for agricultural and grazing activities, whereas Port Curtis supports several major industrial activities. Contaminant levels in dolphins may vary significantly even between nearby regions (Lailson-Brito et al. 2010) and the contaminant profile of the resident dolphin populations may be representative of the difference in concentration and distribution of contaminants found in the local environment (Fair et al. 2010). Geographic differences in contaminant levels in the ERMP survey area between Port Curtis and Port Alma were tested only using biopsy samples of humpback dolphins. Samples were grouped accordingly to the location where the samples were taken as being part of the Port Curtis or Port Alma community. Snubfin dolphins were present only in Port Alma therefore geographic differences could not be tested for this species.

The concentrations of OCs are known to also vary significantly between sexes, less so than those of heavy metals which appear to be more correlated with age. However, age of the dolphins could not be estimated in this project. The sex was determined following protocols described in section 3.2.2. Differences between sexes could be tested only for snubfin dolphins, whereas the large majority of the samples collected from humpback dolphins were female.

Due to the small sample size and expected high variability in contaminant levels among individuals, non-parametric tests (Man-Whitney U Test = MWW; Kruskal-Wallis Test = KW) were used to assess differences in contaminant levels among group. Temporal variation in DDTs, HCB and

ΣPCBs was investigated by comparing results from samples collected in 2010–2011 from the ERMP survey area, (i.e. seven snubfin dolphins and seven humpback dolphins from Port Alma and five humpback dolphins from Port Curtis) with results from this study. Samples from 2010–2011 were collected, stored and analysed in following the same protocols applied in this study (Cagnazzi et al. 2013a). Contaminant levels were also compared with those of similar species available in the scientific literature and with available thresholds.

4.3 Results

4.3.1 Organochlorines

A total of 35 samples (18 snubfin dolphins and 17 humpback dolphins) were analysed for PCBs, DDTs and HCB. All samples of snubfin dolphins were collected in Port Alma. Using standard genetic protocols for sexing, it was established that nine samples were male dolphins and nine were female (Table 14). Of the 17 samples of humpback dolphins, 11 were collected in Port Curtis and six in Port Alma, all samples but two were female.

The sum of all PCBs (ΣPCBs) was highly variable, ranging from 516 (HD-21164) to 222,511 ng/g lw (HD-21264) (Table 14). DDT levels were also very high and ranged from 1,552 ng/g lw in HD-21264) to 74,195 ng/g lw in HD-21271 (Table 14). In contrast, HCB was found generally at low levels (Table 14). No significant difference was found in organochlorine levels between male and female snubfin dolphins (ΣPCB: MWW = 43,000, *p*-value = 0.8; DDTs: MWW = 38,000, *p*-value = 0.8; HCB: MWW = 59,000, *p*-value = 0.1). Not enough male samples were available to statistically compare sexes in humpback dolphins.

No evidence for geographic difference in organochlorine levels was found between samples of humpback dolphins collected in Port Alma and Port Curtis (ΣPCB: MWW = 26,000, *p*-value = 0.5; DDTs: MWW = 28,000, *p*-value = 0.6; HCB: MWW = 30,000, *p*-value = 0.8). Significantly higher concentrations of DDTs and HCB where found only when samples of humpback dolphins from Port Alma were compared to samples of snubfin dolphins from the same area (DDTs: MWW = 95,000, *p*-value = 0; HCB: MWW = 84,000, *p*-value = 0.04) (Table 14). Although ΣPCBs were not significantly different, median values were also substantially higher in humpback dolphins (ΣPCB: MWW = 68,000, *p*-value = 0.5) (Table 14). Of great concern, one female humpback dolphin in Port Alma (HD-21263) had ΣPCBs values among the highest found in published literature and exceeded all available threshold levels for PCBs (222,511 ng/g lw) (Tables 14 and 15). Descriptive statistics for the 30 PCB congeners analysed, DDTs and HCB in biopsy samples of humpback and snubfin dolphins, summarised with mean and range values in lw and ww are presented in Table A.12. DDTs

and PCBs were more abundant than HCB (Table 14). The metabolite pp'DDE was the principal component of DDTs (Figure 18).

The levels of DDTs, HCB and ΣPCBs found in samples collected in this study were compared to levels found in samples collected in the ERMP survey area in 2010–2011 (Table 15). The levels of DDTs, HCB and ΣPCBs recorded in this study for snubfin dolphins were significantly higher than those recorded in 2010–2011 (snubfin dolphins ΣPCBs: MWW = 119,000, *p*-value = 0.00; DDTs: MWW = 124,000, *p*-value = 0.00; HCB: MWW = 113,000, *p*-value = 0.00). Significantly higher DDTs, HCB and ΣPCBs levels were found in biopsy samples of humpback dolphins collected in Port Alma in 2014–2016 compared to those collected in 2010–2011 (ΣPCB: MWW = 37,000, *p*-value = 0.02; DDTs: MWW = 41,000, *p*-value = 0.00; HCB: MWW = 113,000, *p*-value = 0.00). Significantly higher DDTs and HCB levels were also found in biopsy samples of humpback dolphins collected in Port Curtis in 2014–2016, while ΣPCBs levels were similar between sampling seasons (ΣPCB: MWW = 40,000, *p*-value = 0.18; DDTs: MWW = 50,000, *p*-value = 0.00; HCB: MWW = 53,000, *p*-value = 0.02) (Table 15).

Table 14 Descriptive statistics for total PCBs, DDTs and HCB in biopsy samples of humpback and snubfin dolphins collected in the ERMP survey area. Results are presented using mean ± standard error and range (minimum and maximum values). Values are presented in ng/g lw.

Contaminants	ΣPCBs	DDTs	HCB
Snubfin dolphin from Port Alma by sex			
Male (9)	15,975±2,530 (8,059–29,662)	22,548±4,137(10,665–50,648)	88±26 (36–293)
Female (9)	16,458±2,783 (7,465–33,886)	21,786±3,091(6,371–40,565)	56±14 (22–53)
Humpback dolphins for both sex by site			
Port Alma (6)	51,170±34,323 (9,254–222,511)	37,490±4,499(26,402–53,344)	166±43 (32–307)
Port Curtis (11)	16,209±2,501 (516–32,527)	32,930±5,568(1,552–74,195)	145±32 (5–402)

In the table ΣPCBs = 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; DDTs = the sum of the op' and pp', forms of DDT, DDD and DDE.

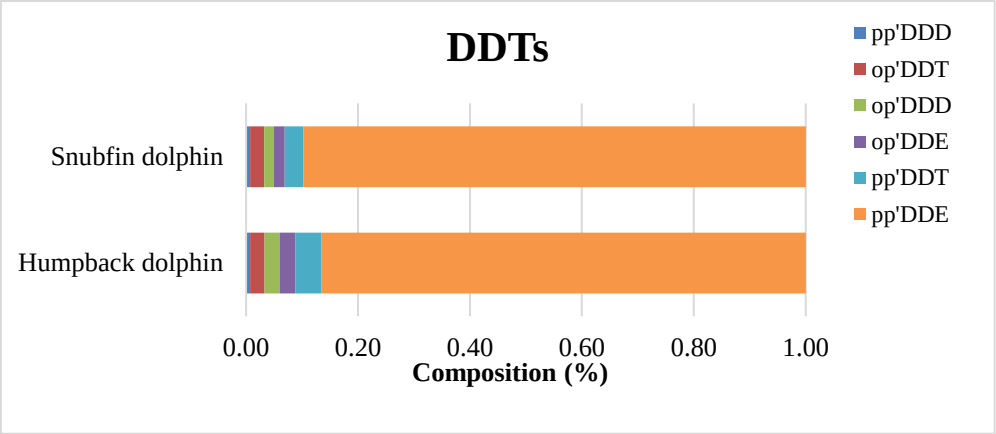

Figure 18 Composition of the two isomers op′ and pp′ of DDT, DDD and DDE in biopsy samples of humpback and snubfin dolphins collected from the ERMP survey area.

Table 15 Comparisons between average values for total PCBs, DDTs and HCB in samples of humpback and snubfin dolphins, collected in the ERMP survey area in 2010–2011 versus those collected in the same area in 2014–2015. Values are expressed in ng/g lw and presented using mean SE = standard error, min = minimum, and max = maximum, sd = snubfin dolphins, hd = humpback dolphins.

Species by site		**sd in Port Alma**		**hd in Port Alma**		**hd in Port Curtis**	
Sampling periods		**2010–11**	**2014–15**	**2010–11**	**2014–15**	**2010–11**	**2014–15**
Contaminants	Sample size	7	18	7	6	5	11
HCB	Mean	23	72	9	166	12	145
	SE	7	15	6	43	9	32
	min	0	22	0	32	0	5
	max	47	293	45	307	47	402
ΣPCBs	Mean	5,008	16,216	5,579	51,750	23,890	16,209
	SE	1,496	1,825	2,635	34,323	17,421	2,501
	min	1,382	7,465	776	9,254	4,006	516
	max	12,280	33,886	19,135	222,511	93,522	32,527
ΣDDTs	Mean	3,774	22,167	3,319	37,490	4,056	32,930
	SE	1,292	2,507	2,168	4,499	735	5,568
	min	178	6,371	491	26,402	2,438	1,552
	max	9,356	54,648	16,073	53,344	6,163	74,195

4.3.2 Heavy metals

A total of 22 skin samples of snubfin dolphins (11 females, 10 males and one unknown), 10 humpback dolphins from Port Curtis (eight females, two males) and seven from Port Alma (six females and one male) were analysed for heavy metals. The profile of the four major elements was the same for both species, with some variation in the profile evident for the heavy metals found at lower concentrations. For example, Hg in snubfin dolphins was the fifth highest concentrated element but in humpback dolphins was only the ninth (Figure 19). Ag was not included as it was always below the detection level of 1 mg/kg.

No significant difference was found in the concentration of heavy metals in skin samples of humpback dolphins from Port Alma (n = 7) and Port Curtis (n = 10) (KW *p*-value > 0.3) and between sexes in snubfin dolphins (KW *p*-value > 0.05). The only significant difference (KW *p*-value ~ 0) was observed between snubfin and humpback dolphins, with snubfin dolphins showing higher concentrations of Zn and Ni (Figure 19).

The large majority of the metal values recorded (Figure 20) for both humpback and snubfin dolphin (294 of the 464 possible combinations) exceeded the maximum epidermal baseline values for free ranging bottlenose dolphins reported in Stavros et al. (2007b) and Bryan et al. (2007). Only Ag, As, Fe and Hg fell largely within expected baseline range values (Figure 20). With the exception of Ag and Fe, all the heavy metals tested in this study were largely above the concentration of the same element found in humpback dolphins from Moreton Bay in Australia (Table 16). For most elements, the values recorded in the ERMP survey area were also higher than those recorded in Indo-Pacific humpback dolphins (*Sousa chinensis*) from Hong Kong and Xiamen in China (Ramu et al. 2005, Wu et al. 2013)

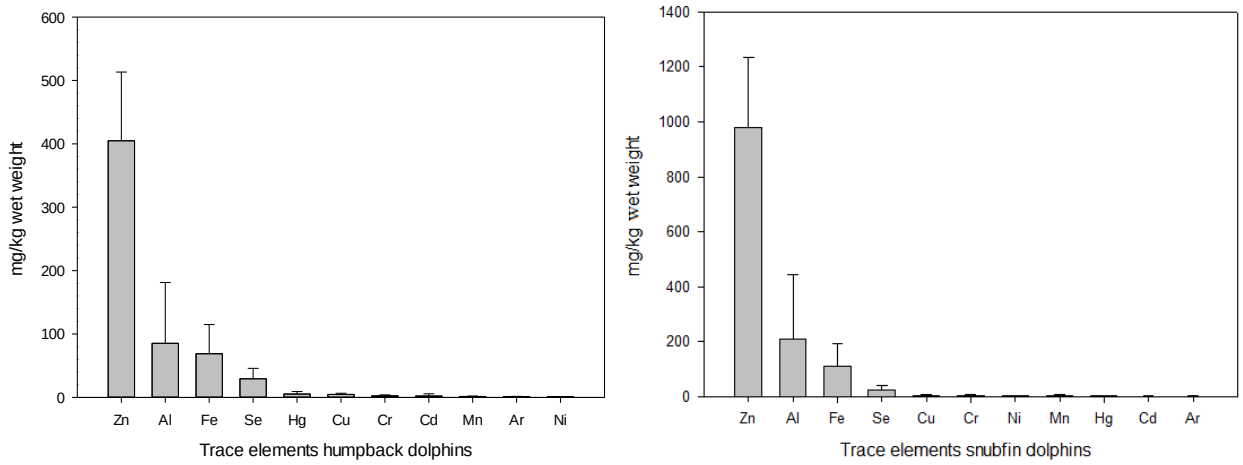

Figure 19 Concentrations and profiles of non-essential microelements (Zn, Fe, Se, Cu, Cr, Mn) and non-essential elements (Pb, Cd, Ag, Hg, Al) in epidermis samples of humpback (left) and snubfin (right) dolphins.

Table 16 Minimum-maximum ranges for elements (all values were given in mg/kg ww, values marked with "*" are in dry weight) in snubfin and humpback dolphins from this study compared to humpback dolphins from Moreton Bay and Indo-pacific humpback dolphins from different regions. DL = detection limit, na = not available.

Heavy metals	This study	Queensland (Australia) (Weijs et al. 2016)	Hong Kong (China) (Parsons 2004)	Xiamen (China)
Cadmium (Cd)	0.01–13.23	< DL–7	0.06–0.029	na
Zinc (Zn)	245–1,373	26.57–258.24	2–18.24*	7.01–40.5
Selenium (Se)	8.71–72.11	2.04–9.57	< 0.46–15.64*	na
Arsenic (As)	<0.5–3.7	0.2–0.7	< 0.35–18.32*	na
Chromium (Cr)	0.65–15.29	0.15–0.27	< 0.35–2.68*	na
Copper (Cu)	1.86–10.66	1.12–1.47	7.03–23.61*	0.73–0.87
Nickel (Ni)	0.41–9.57	< DL–0.15	< 0.30–1.16*	na
Aluminium (Al)	< DL–921.16	< DL–51.83	na	na
Manganese (Mn)	0.25–13.03	0.47–2.12	na	na
Iron (Fe)	14.85–328.33	5.80–229.17	na	na
Silver (Ag)	< DL	< DL	na	na
Mercury (Hg) hd	1.67–15.72	na	na	0.117–11.5
Mercury (Hg) sd	< DL–7.22	na	na	0.117–11.5

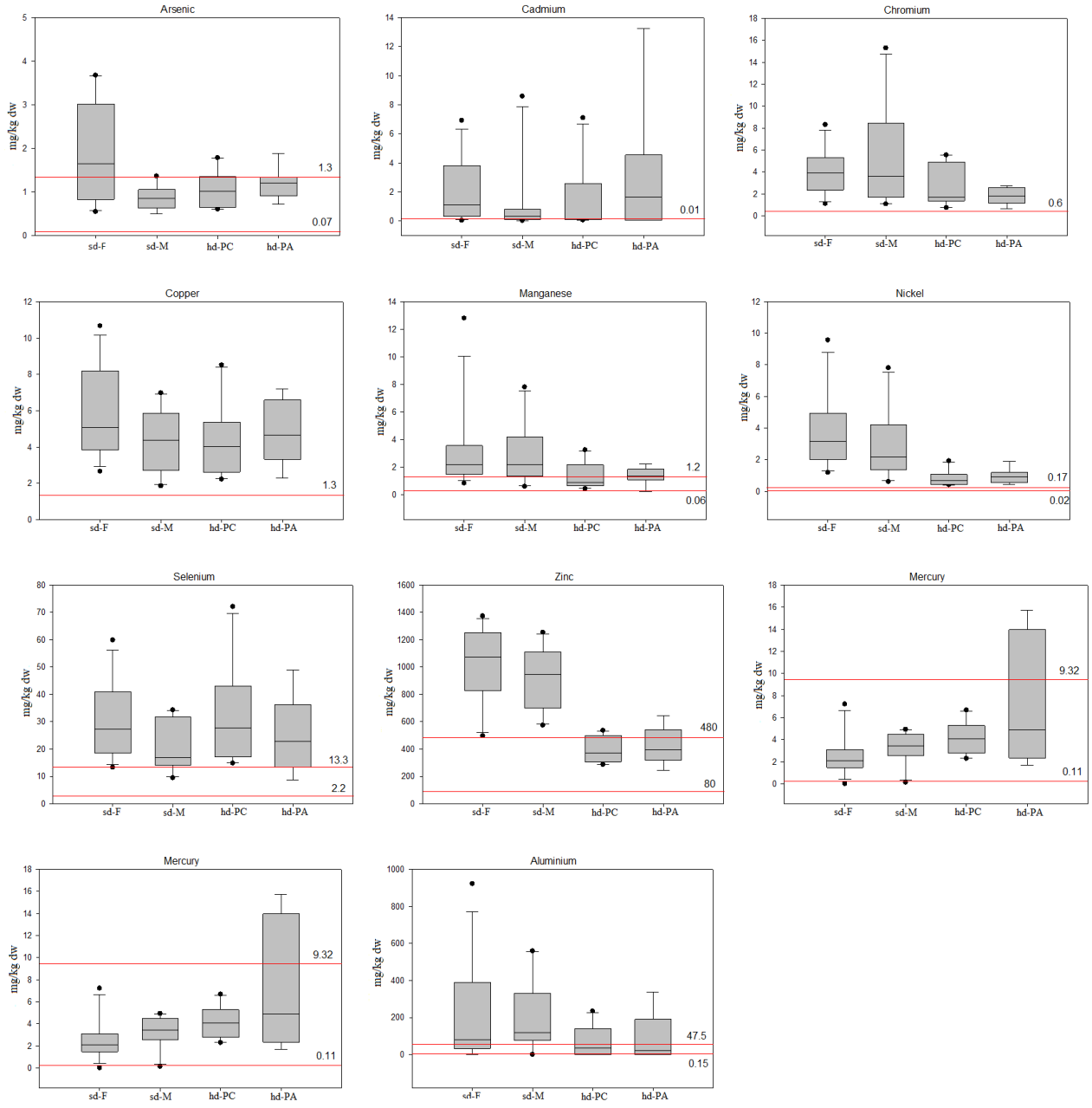

Figure 20 Concentrations of heavy metals in the skin of humpback and snubfin dolphins. Red lines showed the upper and lower values reported for epidermal baseline concentrations in bottlenose dolphins (Bryan et al., 2007; Stavros et al., 2007). In the figure sd = snubfin dolphin, hd = humpback dolphins, PC = Port Curtis, PA = Port Alma, F = female, M = male, dw = dry weight.

4.4 Discussion

4.4.1 Organochlorines

There was no evidence for significant differences in concentrations of organochlorine contaminants in humpback and snubfin dolphins between sampling sites and sexes. However, some differences may be confounded by other biological parameters that cannot be controlled. Exposure levels and accumulation of contaminants can vary between individuals as a result of differences in age, sex,

reproductive history, nutritive condition, health and diet (Aguilar et al. 1999, Yordy et al. 2010). For example, in males, accumulation of some of the more persistent OCs continues through life but, in females, concentrations decline with reproductive activity, through transfer across the placenta and via lactation. The large majority (about 80%) of OCs body burdens are transferred through lactation to the first-born and substantially less to the following calves, but the concentration of OCs transferred varies depending to calf survivorship (Reddy et al. 2001). In cetaceans individual variation in blubber organochlorine concentrations has been linked also to loss of nutritional status and to the presence of infectious diseases (Jepson et al. 2005). These factors can rarely be investigated when samples are remotely collected from free ranging dolphins especially in elusive species such as the snubfin and humpback dolphin.

∑PCBs and DDTs were found in significantly higher proportions than HCB. HCB was detectable in all samples but the concentrations were very low (5.6–402.4 ng/g lw), similar to what has been reported in other studies (Stockin et al. 2010) and not considered dangerous to dolphin health. These results are in accordance with the higher values of PCBs and DDTs than HCB found in the Great Barrier Reef, particularly near port facilities and in the proximity of rivers where samples for this study were taken (Haynes and Johnson 2000, Van Oosterom et al. 2010, Kroon et al. 2012a). ΣPCBs detected in this study were within the range of those reported from stranded humpback dolphins from Moreton Bay in southern Queensland and Indo-Pacific humpback dolphins from south east Asia (Table 17). However, comparisons among ΣPCBs across different studies must be done with caution due to differences in the number and types of PCBs congeners investigated (Table 17).

Blubber total PCB threshold concentration for adverse health effects in all marine mammals of 17,000 ng/g lw was proposed based on experimental studies on PCB-induced immunological and reproductive effects in mammals (Kannan et al. 2000). Among the 35 samples analysed for PCBs, seven snubfin dolphins and nine humpback dolphins (Port Curtis = 6 and Port Alma = 3) exceeded the threshold value. This is a substantially larger proportion (68%) compared to the number of samples (n = 4, 15%) that exceeded the threshold level in 2010–2011 (Cagnazzi et al. 2013a). Additionally, one humpback dolphin from Port Alma had ΣPCBs levels higher (ΣPCBs = 222,511 ng/g lw) than those associated with carcinoma (ΣPCBs = 77,000 ng/g lw) in California sea lions (*Zalophus californianus*) (Ylitalo et al. 2005). Six female snubfin dolphins and 11 female humpback dolphins (Port Alma = 3, Port Curtis = 8) showed ΣPCBs above the threshold value (ΣPCBs = 11,000 ng/g lw) related to foetal and neonatal mortality associated with maternal PCBs exposure (Schwacke et al. 2012). Of these, three female snubfin dolphins (ΣPCBs Range = 20,470–33,886 ng/g lw) and seven female humpback dolphins (three in Port Alma and four in Port Curtis)

(ΣPCBs Range = 18,761–222,511 ng/g lw) showed ΣPCBs values above those associated with impaired reproduction in adult female harbour porpoises (*Phocoena phocoena*) (ΣPCBs = 18,500 ng/g lw).

Table 17 Comparison of mean and maximum levels of PCBs (ng/g lw) found in the genus *Sousa* and *Orcaella*. In the table #PCBs = number of PCBs congeners analysed in the study, NCO = number of congeners overlapping with this study, hd = humpback dolphin, ihd = Indo-Pacific humpback dolphin (*Sousa chinensis*), sd = snubfin dolphin, id = Irrawaddy dolphin (*Orcaella brevirostris*), na = not available.

Study reference	Species	Location	#PCBs	NCO	Mean	Max
This study	hd	ERMP survey area	27	27	28,753	222,511
Weijs *et al.*, 2016	hd	Moreton Bay	19	6	73,701	370,011
Gui *et al.*, 2014	hd	Pearl River	19	5	1,790	na
Wu *et al.*, 2013	hd	Pearl River	27	10	22,023	86,415
Karuppiah *et al.*, 2005	hd	India	11	7	2,218	na
Minh *et al.*, 1999	ihd	Hong Kong	All	3	54,872	154,838
Minh *et al.*, 2000	ihd	Hong Kong	73	25	67,391	108,695
Chou *et al.*, 2004	ihd	Taiwan	19	9	290	na
Kajiwara *et al.*, 2006	ihd	Hong Kong	na	na	45,000	83,000
	ihd	India	na	na	2,000	2,600
Ramu *et al.*, 2005	ihd	Hong Kong	na	na	31,406	83,000
This study	sd	ERMP survey area	30	30	16,216	33,886
Kannan *et al.*, 2005	id	India	na	na	176	390

DDT concentrations are directly comparable to other studies. DDT levels in humpback and snubfin dolphins from the ERMP survey area (28,177–74,195 ng/g lw) are significantly higher (non-overlapping intervals) than those recorded in stranded humpback dolphins from Moreton Bay (1,800–17,000 ng/g lw). Compared to Indo-Pacific humpback dolphins from south-east Asia, the values reported in this study were substantially lower than results from Hong Kong and Pearl River (51,000–471,000) but similar to Zhuhai, Xiamen (Leung et al. 2005, Ramu et al. 2005, Gui et al. 2014). Values were also lower compared to Irrawaddy dolphins from India (29,000–190,000 ng/g lw) (Kannan et al. 2005). Among DDTs, p,p′-DDE accounted for at least 80% of all metabolites. The high p,p′-DDE/ΣDDTs ratio (ratio = 0.91–0.94) confirms that these contaminants are derived from the historical use of these pesticides. A total of 33 of the 36 samples showed p,p′-DDE values higher than those associated with inhibition of transcriptional activity of androgen receptors in mammalian cell cultures (6,890 ng/g lw) (Kelce et al. 1995) and within the range of values (1,430–58,900 ng/g lw) associated with decreased proliferative responses of lymphocytes in free ranging bottlenose dolphins (Lahvis et al. 1995) and splenocytes (spleen white blood cells) in beluga whales (Guise 1998). None of the samples collected in 2010–2011 showed DDE values above the levels that may result in adverse health effects on dolphins (Cagnazzi et al. 2013a).

The DDTs/∑PCBs ratio is generally used to characterise the contributions of pollutants from agricultural and industrial sources to marine mammals (Aguilar et al. 1999, Lailson-Brito et al. 2010). In the present study, the average ratio DDTs/∑PCBs was 1.8, compared to an average of 0.5 recorded in 2010–2011 (Cagnazzi et al. 2013a). This suggests a substantial increase in the input of agriculture associated contaminants that have reached the coastal waters in the last five years, which is also suggested by the significant increase in HCB. In contrast, ΣPCBs remained at similar levels to those recorded in 2010–2011(Cagnazzi et al. 2013a).

Even though the use of PCBs, DDTs and HCB in Australia has been banned since the late 1970s, they remain a common contaminant in the environment due to their stable nature and limited mobility (Tanabe and Tatsukawa 1983, Stemmler and Lammel 2009). Furthermore, PCBs will continue to be produced as combustion by-products and released during the recycling of materials and building demolitions (National Pollution Inventory, accessed 12 January 2018). DDT and its metabolites were the second most commonly detected pesticide in samples collected from irrigation channels in Queensland catchments (Müller et al. 2000).

Land-based run-off is recognised as one of the most significant threats to the long-term health and resilience of the Great Barrier Reef (GBRMPA outlook report, 2014). The Fitzroy catchment is among the regions that generate the largest loads of total suspended solids released in to the Great Barrier Reef lagoon (2,900 ktonnes/year) (Kroon et al. 2012b). In the last seven years this region has experienced four major flooding events (2010–2011, 2013, 2015, and 2017) and cyclones (Yasi 2011, Oswald 2013, Marcia 2015 and Debbie 2017) compared to an average of one major flooding event re-occurrence every six years before 2011 (Lough 2007). Throughout this extended period of extreme weather conditions, Queensland has experienced intense rainfall (BOM 12) followed by long periods of severe drought. The marked increase in river discharge coincides with an increased influx of sediments, nutrients and other known contaminants into the coastal waters from the adjacent catchments (Thompson et al. 2014). OCs contaminants can be absorbed into suspended soil and sediment particles, which accumulate, acting as sinks and long-term sources. They have the ability to be remobilised in marine ecosystems through many pathways including atmospheric transport, riverine inputs, floods and dredging. These contaminants become available to dolphins through the food chain, for example DDT levels from Hong Kong humpback dolphins have been recorded in concentrations up to 212 times higher compared to the residues in the prey fishes (Gui et al. 2014).

Therefore, the significant increase in the levels of PCBs, DDTs and HCB detected in biopsy samples collected in 2014–2016 compared to those collected in 2010–2011 could be explained by an increase in various contaminants transported from the local catchment area to the Great Barrier

Reef lagoon through river run off and associated flooding events. Further, because of their hydrophobic properties, OCs tend to strongly partition to the sediments, which act as their temporary or long-term sinks (Jönsson et al. 2003). On the other hand, resuspension process in estuarine and near-coastal environments may act as a source of OCs to the overlying water column (Eggleton and Thomas 2004).Therefore it is plausible that all the activities associated with WBDDP, including dredging may have facilitated the re-mobilization within the environment of OCs contaminants retained into the sediments of Port Curtis.

4.4.2 Heavy metals

Heavy metals fall into two broader categories of either essential elements (Zn, Cu, Cr., Se, Ni, Al) or non-essential (Hg, Cd, Ar, Ag, Pb). Essential trace elements are part of protein complexes (metalloproteins), that are required for enzymatic activities, and can play structural roles in connective tissue and cell membranes. Non-essential trace elements are considered toxic and are not required for physiological processes (Bryan et al. 2007). Essential trace elements can also be toxic when they are present at excessive levels (Lavery et al. 2009). Heavy metals can be absorbed by marine mammals from the atmosphere through the lungs, absorption through the skin, across the placenta before birth, via milk through lactating, ingestion of sea water and ingestion of food. Among these, bioaccumulation through the food chain is the major route for heavy metal contamination for marine mammals (André et al. 1990, Augier et al. 1993, Law 1996).
The factors affecting the concentration of heavy metals in marine mammals and the link between the concentrations of each element in the various tissues are still poorly understood (Aubail et al. 2013). Therefore, concentrations of heavy metals in internal organs cannot be extrapolated from the values observed in skin and blubber (Weijs et al. 2016). Nevertheless, the skin in cetaceans is a metabolically active tissue which reflects recent dietary inputs and therefore provides more indicative information on recent exposure and the geographic source of the metals compared to internal organs (Aubail et al. 2013).
In this study, the epidermis of 39 samples (22 snubfin dolphins and 17 humpback dolphins) was used to assess the concentration of eight essential elements (Zn, Cu, Cr, Se, Ni, Al, Mn, Fe) and four non-essential elements (Hg, Cd, Ar, Ag). The large majority of samples analysed in this study exceeded the upper baseline values established from biopsy samples collected from free ranging bottlenose dolphins (Bryan et al. 2007, Stavros et al. 2007a). The levels of heavy metals recorded in this study are also higher than those found in similar studies conducted on humpback and snubfin dolphins in Australian and elsewhere (Table 17). This suggests a general enrichment of these contaminants above natural levels. This is also confirmed from a large overlap existing in the

concentrations of Al, Ar, Cd, Fe and Cr between oysters and whelks collected from Port Curtis and the results of this study (Jones et al. 2005).

The sources for heavy metals into Port Curtis and The Narrows are numerous and well documented (Angel et al. 2012, Jones et al. 2014). The Port of Gladstone is an international trade port, coal exports accounted for 70.8% of total port throughput, followed by alumina at 23.8% and a variety of other products including cement, petroleum (www.gpcl.com.au). The town of Gladstone also supports major industries such as alumina refineries and smelter, cements plants and shale oil mines. Extensive commercial and recreational fishing and boating activities occurred throughout Port Curtis. For instance, Cu and Zn are discharged into waters of the Calliope River and the Yarwun trade waste outlet at Fisherman's Landing, by industries such as the Gladstone power station, Boyne Smelter and sewage works (National Pollution Inventory, accessed 16 January 2018). The leachate from reclaimed land containing fly ash from the nearby power station and antifouling paints from boats may also contribute to the higher concentrations of dissolved copper and zinc in the inner harbour (Warnken et al. 2004, Jones et al. 2005). Many trace metals are associated with manganese and iron (hydr)oxides in sediments and may be realised in the environment following the reduction of manganese and iron (hydr)oxides or through natural geochemical weathering and leaching processes.

Under normal operational conditions most dissolved metal concentrations are significantly higher in Port Curtis compared to the concentrations measured in the adjacent coastal waters (Angel et al. 2010). Cd, Cr, Ni, Cu, Mn and Al were also detected in The Narrows in levels higher than those recorded in reference sites. However, apart from a few exceptions (dissolved Al and As) both dissolved and particulate metals are below the marine water quality guideline trigger values that apply in Australia (ANZECC/ARMCANZ 2000, Angel et al. 2012).

While the occurrence of trace metals in Port Curtis is well documented, there is a limited knowledge surrounding background levels and the potential sources of metals in Port Alma. The Fitzroy Basin is ~140,000 km^2 and comprises of 11 sub-basins. This vast area includes over 40 coal mines which release mine-affected water and sediments into the Fitzroy River Basin. Coal mine-affected waters were identified to be an important source of metals in this region. For example ~ 70% of the dissolved Al data, from both upstream and downstream of coal mine-affected water release points, exceeded the trigger value (55 µg/l, pH >6.5) (Jones et al. 2014). In this area, dissolved Cu, Zi, and Ni also often exceeded the ANZECC/ARMCANZ (2000) toxicant trigger levels. Fertilisers and herbicides are widely used in the Fitzroy River basin and are also a potential source of heavy metals with the potential to reach coastal waters during flooding of adjacent rivers (GBRMPA, 2013).

In Port Curtis, The Narrows and Port Alma Ni, As, Cd, Cr and Mn are naturally found in the sediments (Angel et al. 2012).

Overall, most of the heavy metals analysed in this study are reportedly widespread in the ERMP survey area which could explain the lack of geographical differences detected in skin samples of humpback and snubfin dolphins collected from Port Curtis and Port Alma.

In this study, the heavy metals of major concern based on their concentration and potential effects (threshold values for marine mammals existed only for Hg found here in low levels) were Cd, Cr and Al. Cd is regarded as one of the most toxic metals and for humans, high dietary concentration may result in several severe adverse effects such as spinal deformities and renal dysfunctions. In some individual dolphins, the concentration of Cd (> 10 ppm) and especially Al (>> 50 ppm) were at levels that if also recorded in the blood would affect the functional activity of leukocytes in bottlenose dolphins (Pellissó et al. 2008). Cr was found at levels (mean ~ 1.9 ppm, max ~ 13.3 ppm) known to have cytotoxic and genotoxic effects (1.71–19.6 ppm) in whale skin (Wise et al. 2015). Another potential element of concern was the levels of Zn in snubfin dolphins. Zn is an essential element however it can be toxic in high concentrations (Augier et al. 1993, Lavery et al. 2009). In cetacean skin, Zn has critical functions in pigmentation of skin during the wound healing process, as well as host immunity (Bryan et al. 2007, Stavros et al. 2007b). Zinc is the only heavy metal found in higher concentrations in skin than internal tissues. In stranded humpback dolphins from Moreton Bay the ratio of skin/liver concentration was about 6.5 (Weijs et al. 2016). If the same skin/liver ratio can be applied to predict the concentration of Zn in the liver of snubfin dolphins, the average levels of zinc in the liver of snubfin dolphins from the ERMP survey area (mean of predicated Zn level in the liver of snubfin dolphins ~ 150 mg/kg ww) would be very close to those associated with renal damage in bottlenose dolphins (178 mg/kg ww) (Lavery et al. 2009). However, data on Zn concentrations in the genus *Orcaella* is not available. Furthermore, the significant difference recorded in this study may reflect biological differences between the two species, which also have a substantially different skin colouration (Beasley et al. 2005, Jefferson and Rosenbaum 2014).

4.4.3 Conclusions

The advantages and disadvantages of using biopsy samples collected from live wild dolphins for contaminant assessment studies is well documented (Méndez-Fernandez et al. 2016). The small sample volumes obtained from biopsies limits the analysis to few contaminants. In particular, in this study the concentrations of PCBs, DDTs, HCB was tested in the blubber of 35 samples and suite of 12 heavy metals in the epidermidis of 37 samples.

The accumulation of organic compounds and trace elements in dolphins is regulated by many factors, such as nutritional conditions, health, life stage, reproductive status and sex, which are rarely known when studies are conducted on wild dolphins. Contaminant levels may therefore vary significantly among individuals and as a result, differences among populations may be hidden by unknown biological factors. However, this technique provides an invaluable insight into the toxicological status of a wild population of small cetaceans. This is especially true for species like humpback and snubfin dolphins that are extremely difficult to catch, so more detailed health status assessments cannot be conducted.

Despite the sample size of this study (snubfin dolphins = 22; humpback dolphins = 17), it represents at least 10% of the estimated adult population of each species (snubfin dolphins = 140; humpback dolphins = 162), and samples were randomly collected from free ranging individuals known to be long-term residents in the region. Therefore, these results are considered representative of the toxicological status of these inshore dolphins in the ERMP survey area.

The findings from this study revealed that inshore dolphins of the ERMP survey area are exposed to relatively high levels of pollutants and may be predisposed to infectious diseases as a result of immunosuppression effects caused by high levels of PCBs, DDTs and some metals. Considering the significant increase in PCBs, DDTs and HCB levels over a five year period and that only a fraction of the potentially dangerous anthropogenic contaminants or natural elements could be tested for, these results are likely to underestimate the toxicological risk for inshore dolphins in the ERMP survey area, and along the Queensland coastline. Therefore, large mortality events of dolphins, like that recorded in Port Curtis in 2011, may reoccur in the area if the local marine environment is subjected to substantial changes as a result of natural or anthropogenic factors.

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

5.1 Introduction

Determining the diet of animals facilitates an understanding of their food requirements, interspecific interactions (competition, resource partitioning), functional roles in the ecosystem and how they might respond to environmental and ecological fluctuations in that ecosystem (Bowen and Iverson 2013). Therefore, an understanding of the feeding ecology of wildlife is of critical importance for guiding conservation decisions as well as for understanding key ecological processes that govern ecological communities such as competition and resource partitioning (Parra 2006, Quérouil et al. 2013).

Snubfin and humpback dolphins live in sympatry throughout most of their range in northern Australia, with some populations showing considerable overlap in the use of space (Parra 2006). Such overlap in space use could result in interspecies competition for food and space (Leal and Fleishman 2002), whereby animals compete directly with each other via targeted aggression or indirectly via competition for resources (Kiszka et al. 2011). Such interactions can result in a mutual suppression of success (Oviedo 2007) because aggressive interactions can cause injury or death, and indirect competition reduces resource availability and foraging success (Ritz 1994). Consequently, mechanisms such as resource partitioning are important in promoting the coexistence of sympatric animals (Leal and Fleishman 2002, Parra 2006, Oviedo 2007, Ansmann et al. 2015). Slight differences in habitat preferences and diet appear to be some of the principal factors promoting the coexistence of snubfin and humpback dolphins (Parra et al. 2006b, Parra and Jedensjö 2014). Previous studies have shown that snubfin dolphins in northern Queensland preferred slightly shallower (1–2 m) waters than humpback dolphins (2–5 m), and favoured seagrass meadows much more often than humpback dolphins (Parra et al. 2006b). Stomach content analysis showed the diet of snubfin and humpback dolphins overlapped partially, particularly across the fish taxa consumed by both species (Parra and Jedensjö 2014). However, humpback dolphins appeared to favour fish; while the snubfin dolphin diet also included a large amount of cephalopods (Parra and Jedensjö 2014). While stomach content analysis is valuable in studies of diet

composition, constraints on sample size and limitations associated with stomach content analysis prevent a clear understanding of dietary partitioning between both species being achieved.
Stomach content analysis can be biased due to inherent problems in the sampling regime and prey identification (Pierce and Boyle 1991, Santos et al. 2001). Stomach contents can only be collected from dead stranded animals, which limits sample sizes as often stomachs from dead stranded individuals are empty (Barros et al. 2004, Matley et al. 2015). Stranded animals may also have been engaged in abnormal feeding behaviour before stranding due to illness (Owen et al. 2011, Parra and Jedensjö 2014). These factors may misrepresent the actual diet of the animal. Stomach contents are also biased towards hard parts such as otoliths and beaks which are resistant to digestion (Browning et al. 2014c) and may result in over-estimation of the importance of particular prey such as cephalopods (Bowen and Iverson 2013). Alternatively, erosion of hard parts may result in misidentification of prey species (Dunshea et al. 2013).
In recent years, several molecular methods have been developed to study animal feeding ecology that are applicable to marine mammals which have overcome many of these limitations, including the analysis of stable isotopes of carbon and nitrogen (Ben-David and Flaherty 2012). Carbon and nitrogen isotopes are most useful for studying the feeding ecology of animals because they are incorporated primarily through lipids and carbohydrates, and dietary proteins respectively (Newsome et al. 2010, Caut et al. 2011, Ben-David and Flaherty 2012). Stable isotope analysis can provide information on the feeding ecology of the target species, and interspecific interactions and trophic level (Kelly 2000, Newsome et al. 2010), and target animals do not require re-capture (Zeppelin et al. 2015). Stable isotopes also allow us to investigate the feeding behaviour of an animal over multiple weeks prior to the sampling event (Browning et al. 2014c) and should therefore provide a fair representation of normal feeding behaviour (Owen et al. 2011).
Carbon isotope ratios can differ in a marine system based on temperature, levels of dissolved CO_2 and the rate of photosynthesis in the system (Ben-David and Flaherty 2012). As a result, there are traceable differences in carbon ratios between pelagic and benthic environments and offshore and inshore environments (Kelly 2000, Ben-David and Flaherty 2012, Browning et al. 2014c, Liu et al. 2015) which can assist ecologists in determining the habitat an animal has been feeding in. Such analysis has been used to show that long-finned pilot whales (*Globicephala melas*) in northwest Iberia feed mainly in coastal environments, while pilot whales from Scotland revealed more oceanic preferences (Monteiro et al. 2015). These findings were supported by stomach content analysis of dead stranded animals and isotope mixing models (Monteiro et al. 2015). Similarly, a study investigating habitat and resource partitioning in multiple delphinid species along the coast of South Africa used carbon isotope ratios to show that striped dolphins (*Stenella coeruleoalba*) preferred

offshore habitats, compared to long-beaked common dolphins (*Delphinus capensis)* and Indo-Pacific humpback dolphins (*Sousa chinensis*), which showed a preference for more inshore habitats (Browning et al. 2014b).

Trophic position can also be measured using nitrogen isotopes since nitrogen is enriched predictably with each trophic step (2–5‰) (DeNiro and Epstein 1978, Kelly 2000, Crawford et al. 2008, Bowen and Iverson 2013), due to preferential incorporation of the heavier form of nitrogen into tissue (Kelly 2000). Using stable isotope analyses from dolphin teeth, it was found that sub-populations of common bottlenose dolphins along the Victorian coast fed at different trophic levels. Those inhabiting Port Phillip Bay displayed elevated nitrogen values of N17‰ whereas dolphins inhabiting the Gippsland Lakes only exhibited nitrogen values of N15.5‰, suggesting the Port Phillip Bay population fed on prey species of a higher trophic level (Owen et al. 2011). Similarly, nitrogen isotope ratios of sympatric sub-populations of common bottlenose dolphins in the Indian River Lagoon in Florida showed that dolphins in Saint Louie estuary fed at higher trophic levels than animals in any other section of the Indian River lagoon (Browning et al. 2014a).

In this study, stable isotopic composition (carbon and nitrogen) to assess feeding ecology of humpback and snubfin dolphins in the ERMP survey area. Further, differences in stable isotopic composition between snubfin and humpback dolphins were used to investigate interspecific resource partitioning, isotopic niche width and overlap of niche space. The null hypothesis was that (1) snubfin and humpback dolphins are feeding at similar trophic levels and that this would be reflected in similar $\delta^{15}N$ isotopic values, (2) snubfin and humpback dolphins have similar trophic niches that would be reflected by overlap of niche spaces, and (3) snubfin dolphins $\delta^{13}C$ stable isotopic composition would reflect they foraged in more nearshore, demersal and/or benthic habitats, while humpback dolphins foraged in relatively more pelagic, offshore habitats.

5.2 Methods

5.2.1 Sample collection

Sampling protocols are described in paragraph 3.2.1 and 4.2.1.

5.2.2 Sample preparation and stable isotope analysis

Preparation of skin samples followed standard protocols for stable isotope analysis (Browning et al. 2014c). 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 60ºC for 24

h 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 sec to ensure proper mixing (Post et al. 2007). Lipid-extracted samples were then placed in a centrifuge for 5 min at 1000 rpm. The remaining solution was discarded and samples were again placed in a drying oven at 60°C for 24 h 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 (IRMS). 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.

5.2.3 Statistical 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 SPSS statistics 24 (IBM). Tests revealed normality for all isotopes and species except for $\delta^{13}C$ for humpback dolphins, which was not normally distributed (p-value = 0.019). Equality of variance between species (p-value = 0.483 for carbon and 0.400 for nitrogen) was satisfied, therefore, non-parametric tests (Kruskal-Wallis) were used to investigate interspecific differences in isotope content. Significance level was set at 95% for all statistical tests.

To reveal key aspects of the trophic ecology of snubfin and humpback dolphins group metrics were calculated using the following population quantitative metrics derived from stable isotope data (Layman et al. 2007, Jackson et al. 2012):

1. Total area (TA), which is a measure of the total amount of niche space occupied by a species in $‰^2$. TA was calculated from a convex hull drawn around the most extreme data points on an isotope $\delta^{13}C$–$\delta^{15}N$ bi-plot.

2. TA is sensitive to differences in sample size because the area can only increase as new data points are added. As a result, the TA is biased towards higher sample sizes. Therefore, the corrected version of the standard ellipse area (SEA_c) was used as a measure of the mean core area (40%) of each species isotopic niche (Jackson et al. 2011, Browning et al. 2014c). The standard ellipse is calculated using the covariance matrix
$$\left(\Sigma = \begin{bmatrix} \sigma_x^2 & cov(x,y) \\ cov(y,x) & \sigma_y^2 \end{bmatrix}\right)$$
to determine size and shape and the mean of x and y to determine the location.
3. Overlap of SEAc, represented as a percentage of the niche space a group shares with another select group, as a quantitative measure of dietary similarity among species.
4. $\delta^{15}N$ range (NR_b), which is the difference between the highest and lowest $\delta^{15}N$ values of each species. NR_b provides information on the vertical structure of the species and represents the trophic diversity of the species.
5. $\delta^{13}C$ range (CR_b) is a measure of the difference between the highest and lowest $\delta^{13}C$ values of each species. CR_b provides an estimate of the variability of food sources consumed.
6. Mean distance to centroid (CD) is the mean Euclidean distance of each individual of a population to the $\delta^{15}N$–$\delta^{13}C$ centroid, where the centroid is the mean $\delta^{15}N$–$\delta^{13}C$ value for all species in the food web. CD provides an estimate of overall dietary diversity.
7. Mean nearest neighbour distance (MNND) is the average nearest-neighbours Euclidean distance between an isotopic coordinate relative to all other coordinates within a species. MNND provides an estimate of species packing and shows how similar or dissimilar the members of a population are to one another.

All metrics were calculated using the Stable Isotope Bayesian Ellipses (SIBER) package (Jackson et al. 2011) in R (R Core Team 2013). The overlap function in the R package SIAR (Parnell and Jackson 2013) was used to determine the isotopic niche overlap between species (Jackson et al. 2011).

5.3 Results

Stable isotopes were extracted from 31 skin samples of adult snubfin and 23 of humpback dolphins. Snubfin dolphin $\delta^{13}C$ values varied from -18.2 to -13.9 (mean ± SD = -15.910 ± 0.845); and $\delta^{15}N$ values varied from 8.9 to 13.3 (mean ± SD =11.160 ± 1.003). No significant difference was found in $\delta^{13}C$ and $\delta^{15}N$ values in samples of humpback dolphins from Port Alma plus and Port Curtis plus Rodds Bay ($\delta^{13}C$: MMW = 27, *p*-values = 0.31; $\delta^{15}N$: MMW = 41, *p*-value = 0.96). Therefore, in

the following analyses all samples of humpback dolphins were considered as single group. Humpback dolphin $\delta^{13}C$ values varied from -18.5 to -13.9 (mean ± SD = -16.348 ± 1.151); and δ15N values varied from 9.9 to 13.5 (mean ± SD =11.226 ± 0.879). Snubfin and humpback dolphins showed no significant difference in $\delta^{15}N$ values (KW: df =1, χ^2 = 0.060, *p*-value = 0.806). Small but statistically significant differences in $\delta^{13}C$ composition were detected (KW: df=1, X^2=3.986, *p*-value = 0.046), with snubfin dolphins having a higher mean $\delta^{13}C$ than humpback dolphins (Table 18, Figure 21).

Table 18 Mean values (±SD) of $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) of 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

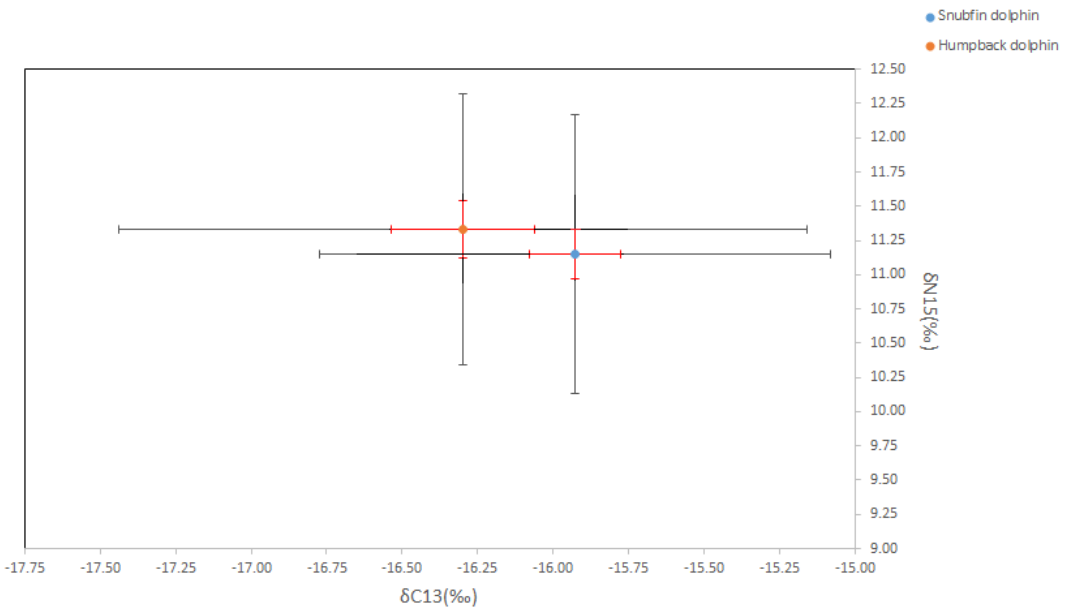

Figure 21 Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) bi-plot showing inter-specific differences in isotope values (mean ±SE and SD) of snubfin and humpback dolphins. Red bars are standard errors and black bars are standard deviations.

The TA occupied by humpback dolphins was slightly greater than snubfin dolphins (Table 19, Figure 22). The mean core area (40%) of each species isotopic niche (SEAc), corroborated that humpback dolphins occupied a slightly greater niche space than snubfin dolphins (Table 19, Figure 22), however, this difference was not significant (*p*-value = 0.694). There was high overlap in SEA_c between both species, with 70.9% of snubfin dolphins SEA_c overlapping with the SEA_c of humpback dolphins (Table 19, Figure 23). Snubfin and humpback dolphins had similar $\delta^{13}C$ range values (snubfin CR = 4.3‰; humpback CR = 4.6‰, Table 19) suggesting that they utilise broadly a

similar range of feeding grounds. $\delta^{15}N$ range values showed a greater range in snubfin (NR = 4.4‰) than humpback dolphins (NR = 3.7‰), indicative of greater variation in the trophic level of their diet (Table 19). Mean distance to centroid (CD) was higher for humpback dolphins (CD = 1.227‰) than for snubfin dolphins (1.078‰) (Table 19), which suggests humpback dolphins have access to more prey species than snubfin. Mean nearest neighbour distance (MNND) of humpback dolphins was higher (MNND = 0.529) than for snubfin dolphins (MNND = 0.369) (Table 19), suggesting that there is greater variation of diet within humpback dolphins.

Table 19 Species level metrics of trophic structure for snubfin and humpback dolphins. TA = Total area, SEA_c = standard ellipse area; CR_b = $\delta^{13}C$ range, NR_b = $\delta^{15}N$ range, CD = mean distance to centroid; MNND = mean nearest neighbour distance.

Species	SEA (‰^{2})	SEAc (‰^{2})	TA (‰^{2})	SEAc Overlap(%)	CR (‰)	NR (‰)	CD (‰)	MNND (‰)
Snubfin	2.624	2.712	12.065	70.9	4.3	4.4	1.078	0.369
Humpback	3.451	3.608	12.715	61.9	4.6	3.7	1.227	0.529

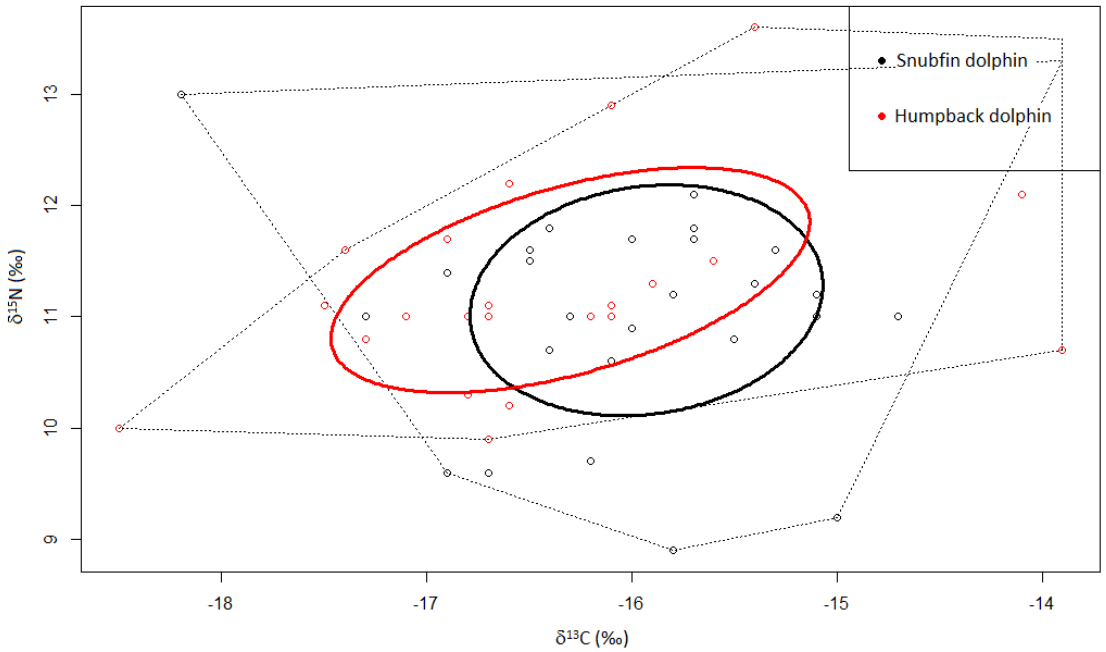

Figure 22 $\delta^{13}C$ and $\delta^{15}N$ stable isotope bi-plot showing the core isotopic niche of snubfin and humpback dolphins in the form of standard ellipses corrected for small sample sizes, SEAc. Convex hulls represent the overall niche diversity and encompass all data points.

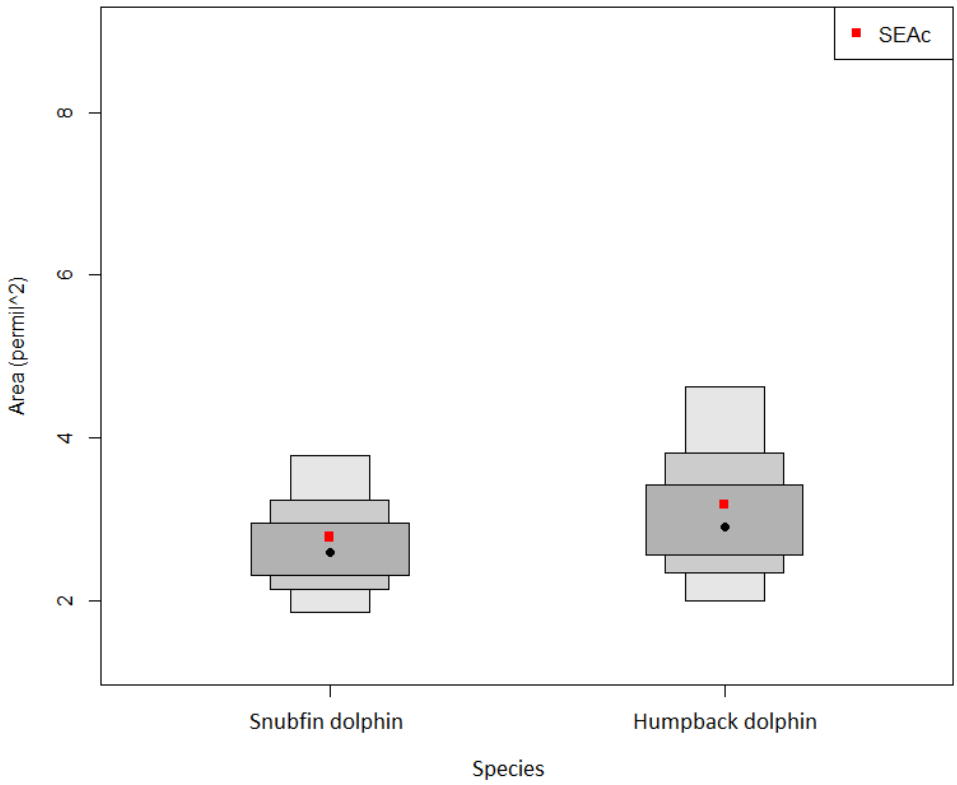

Figure 23 SIAR density plot showing the 95, 75 and 50% confidence intervals of standard ellipses area using Bayesian techniques. The black dots represent the mean standard ellipses area (SEA) for each species and the red squares represent the corrected standard ellipses area (SEA_c).

5.4 Discussion

Among sympatric species of inshore dolphins, interspecific differences in $\delta^{15}N$ and $\delta^{13}C$ isotopic composition are expected to be small, as humpback and snubfin dolphins share similar feeding habitats and preys preferences (Parra 2006, Parra and Jedensjö 2014). The interspecific similarities in $\delta^{15}N$ and differences in $\delta^{13}C$ isotopic composition found in this study suggest 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 $\delta^{13}C$, which is indicative of foraging in more inshore, benthic habitats than humpback dolphins. Our findings are consistent with previous studies, based on space use and stomach content analysis, indicating that both species feed on similar prey (Parra and Jedensjö 2014), but use slightly different habitats (Parra 2006). However, given the small effect size in $\delta^{13}C$ isotopic composition, further analysis including larger sample sizes are needed to confirm differences in foraging preference between snubfin and humpback dolphins indicted in these results.

Stomach content analyses conducted by Parra and Jedensjö (2014) have shown some interspecific differences in consumption of cephalopods, with humpback dolphins relying mainly on fish for food and rarely including cephalopods in their diet compared to snubfin dolphins. Despite these

differences, the fish prey in the diet of snubfin and humpback dolphins overlapped considerably. All fish taxa identified to genus in stomachs of humpback dolphins were also consumed by snubfin dolphins, and the most numerically important fish prey item in the stomach contents of each dolphin was also consumed by the other (Parra and Jedensjö 2014). This is reflected in the similar isotopic niche width metrics (TA and SEAc) and the substantial (≥ 60%) isotopic niche overlap found between snubfin and humpback dolphins, suggesting there is little trophic niche segregation between both species.

Although, SEA_c values overlapped, the isotopic niche width was slightly greater for humpback dolphins suggesting they use a wider range of prey than snubfin dolphins. This was also reflected in the CD and MNND metrics, which were higher for humpback dolphins suggesting greater dietary diversity and greater diet variation within the species. Nitrogen range, however, was higher in snubfin dolphins suggesting a greater variation in the trophic level of their diet. This may be explained by the prevalence of larger amounts of cephalopods in their stomach contents in comparison to humpback dolphins (Parra and Jedensjö 2014).

Many cetaceans, including snubfin and humpback dolphins are considered opportunistic generalized feeders (Browning et al. 2014c, Parra and Jedensjö 2014) meaning they feed on a wide variety of prey species that are readily available. Cephalopods are abundant in shallow water (≤1 m deep) along the Queensland coast (Moltschaniwskyj and Doherty 1995), where snubfin dolphins preferentially forage, and this provides a possible explanation for the greater variation in the trophic level (nitrogen range) of snubfin dolphins diet in comparison to that of humpback dolphins.

According to niche overlap theory, the number of species that can share a similar trophic niche within a community may increase without further competition, with increased food availability (Pianka 1974). Without an abundance of food, animals that live in sympatry are expected to engage in interspecific competition unless there is some form of resource partitioning, whereby animals utilize different habitats or prey on different species (Kiszka et al. 2011). The coastal-estuarine environments along the coast of Queensland are highly productive areas, and as such, may promote co-existence despite a great deal of overlap in the diet of snubfin and humpback dolphins.

Aggressive interactions have been shown to lead to habitat segregation in sympatric bird species (Martin and Martin 2001). Observational data has identified aggressive interspecific interactions between snubfin and humpback dolphins, and has been proposed as one of the reasons for slight differences in habitat selection in snubfin and humpback dolphins (Parra et al. 2006b). Snubfin dolphins may selectively forage in more inshore environments to avoid aggressive interactions with the dominant humpback dolphins. This avoidance is evident by differences in $\delta^{13}C$ composition of snubfin and humpback dolphins and could partially explain the increase in the number of snubfin

dolphins recorded in Port Alma in 2015 which was contemporaneous to a significant drop in the number of humpback dolphins in the same area.

Understanding feeding ecology and habitat preference of coexisting species is necessary to determine species specific requirements and where to direct conservation efforts (Sachot et al. 2003, Browning et al. 2014b). The findings in this study highlight the importance of coastal and estuarine environments for these species. The overall diversity of prey species suggests that these animals may be somewhat resistant to losses in prey abundance of some species, however, the importance of cephalopods for snubfin dolphins' diet is emphasised, based upon increased nitrogen range.

While stable isotope analysis can provide important information on consumer–resource relationships, it is important to acknowledge that overlap in isotopic values of consumers does not necessarily indicate the same feeding habits or diet, as different prey species with similar isotopic signatures may produce similar $\delta^{13}C$ and $\delta^{15}N$ isotope values (Phillips et al. 2014, Santos-Carvallo et al. 2015). Despite these constraints, the findings from this study provide valuable insights into the trophic ecology of snubfin and humpback dolphins and suggest that 1) both species are opportunistic-generalist feeders preying on a variety of fish primarily found in inshore waters, 2) there is little variation in their diet and 3) habitat partitioning could be the primary mechanism for competition avoidance and play a vital role in promoting their coexistence.

6 Conclusions and recommendations

6.1 Main findings

This project has provided substantial new information on abundance, movement patterns, genetics and population structure, feeding preferences and accumulation of common contaminants of humpback and snubfin dolphins within the ERMP survey area. For the current project the ERMP survey area was divided into four different sub-areas (Rodds Bay, Port Curtis, Port Alma and Keppel Sands, East Curtis Island). The majority of sightings of humpback dolphins occurred in Port Curtis (n = 130) followed by Port Alma and Keppel Sands (n = 72) and to a lesser extent in Rodds Bay (n = 46) and East Curtis Island (n = 5, of which two were of the same single individual). All sightings of snubfin dolphins were recorded in Port Alma and Keppel Sands. The Narrows was also identified as an important habitat for humpback dolphins and to a lesser extent for snubfin dolphins. Humpback dolphins where found throughout The Narrows, and one humpback dolphin was observed transiting the Ramsey Crossing from Port Curtis to Port Alma. In contrast, snubfin dolphins were observed only in the northern end of The Narrows. Based on the number of sightings and the geographic patterns of re-sightings of marked individuals, Port Curtis and Port Alma appear to be key areas for the survival of both species in the entire ERMP survey area.

Port Curtis and Port Alma are subjected to different anthropogenic and environmental stressors. The Fitzroy Basin is one of the largest catchments of the Great Barrier Reef and receives groundwater from numerous mining and farming areas which release water and sediments with chronic elevation of potentially toxic compounds (Great Barrier Reef Marine Park Authority 2013). Much of natural vegetation of the Fitzroy Basin has been heavily modified which has resulted in impacts to inshore marine areas from increases in sediment, nutrient and contaminant loads that are transported from the Fitzroy River (Great Barrier Reef Marine Park Authority 2013). Port Curtis is home of one of Australia's largest ports the Port of Gladstone and it is subjected to intensive boat traffic for both recreational and commercial purposes (Jones et al. 2005, Melville et al. 2009). Both areas support a variety of commercial fisheries and recreational fishing.

Based on association patterns and distribution of photo-identified individuals in previous research, humpback dolphins in the ERMP survey area were subdivided into two distinct social units: the Port Alma and Port Curtis sub-populations (Cagnazzi 2010). These results were confirmed in the present study by the analyses of photo-identification data and molecular variance, which showed a very limited movement of individuals between Port Alma and Port Curtis. A significant level of genetic differentiation was identified between samples collected from the two locations, but these results also showed a moderate migration rate in a north-south direction (~ 25% individuals per

generation ~ 20 years). Considering previous knowledge, the evidence provided in this study, and the different threats potentially faced by dolphins in Port Alma and Port Curtis, humpback dolphins in Port Alma and Port Curtis should be considered to be two separate sub-populations (i.e., geographically or otherwise distinct groups in the regional population between which there is little demographic or genetic exchange) for local management and conservation purposes.

For example, in Port Curtis the identification of dolphin's core habitats associated with a community campaign may help to mitigate the negative effects of intensive boat traffic. In Port Alma, slow vessel movement zones or protected areas are likely to provide little benefit to the local sub-population which is primarily affected by floods and poor water quality. In Port Alma, the reestablishment of riparian zones to stabilise and reduce erosion and the amount of sediments deposited from the catchment into the estuary would help improve water quality.

At a regional scale these sub-populations should be considered as a single management unit and efforts should be focused on maintaining the present gene flow by protecting existing corridors including The Narrows. For example, industrial development in The Narrows may affect dolphin movements, further limiting the small but important migration patterns recorded in this study.

Before the flood and the WBDDP in 2010-2011, the number of humpback dolphins using the Keppel Bay region between 2007–2011, which includes Port Alma, was estimated to range between 115 (SE = 7.9, 95%CI = 100–130) in 2007 to 104 (SE = 8.19, 95%CI = 88–120) in 2011 (Cagnazzi 2011). During the same period the number of humpback dolphins in Port Curtis was estimated to range between 84 in 2007 (SE = 5.8, 95%CI = 73–95) to 45 individuals in 2011 (SE = 7.7, 95%CI = 30–61) (Cagnazzi 2013). In 2011, following the large flood and the concurrent commencement of the WBDDP, the number of humpback dolphins in Port Curtis declined significantly by about 40%, while in Keppel Bay a non-significant decline of 9% was observed. The decline in abundance estimates coincided with at least nine humpback dolphins fatalities in 2011 across the ERMP survey area (Meager and Limpus 2014). Based on total adult population estimates from the present study (2014–2016), the number of humpback dolphins in Port Curtis (larger $\widehat{N}_{\text{total}} = 85$, SE = 0.08, 95%CI 71–99) have subsequently returned to their original level prior to 2011.

Humpback dolphins have been sighted throughout the entire Port Curtis area, with no apparent variation in habitat use patterns compared to observations collected before 2011. Analysis of genetic data conducted as part of the present study also did not provide any indication of a recent bottleneck, and the low genetic diversity appears to be a natural characteristic of this species around Australia (Brown et al. 2014b). Considering the short time frame of the population recovery, the observed decline in humpback dolphins in Port Curtis could be partially explained by a temporary shift in the dolphins' distribution. During the three years of this study, several individuals were

resighted between Port Curtis and Rodds Bay. It is therefore possible that during the disturbance period the core group of humpback dolphins normally living in Port Curtis, moved to nearby regions and waited until more suitable conditions were re-established in Port Curtis before they returned. A similar temporal pattern was observed in the decline and recovery of sea grass abundance in Port Curtis (Bryant et al. 2016). Bryant et al. (2016) identified the flood as major driver to the decline in seagrass abundance however the concurrent timing with the WBDDP made it impossible to ascertain what additional impact the WBDDP may have had on sea grass. Similarly, the major drivers of the temporary decline in the number of humpback dolphin using Port Curtis in 2011 remain unknown. Surveys conducted prior to this study were not designed to assess the impacts of natural or anthropogenic factors on humpback dolphins, whereas the current study was conducted after the completion of the WBDDP and few years after the flood.

However recent studies have shown a broad range of negative effects of floods and anthropogenic activities associated to port development in a variety of dolphin species. Variation in distribution, ranging patterns, and relative abundance as result of prolonged, repeated flooding events (Nowacek et al. 2001, Cardoso et al. 2008, Fury and Harrison 2011, Fury and Reif 2012) and anthropogenic disturbance (Jefferson et al. 2009) has been observed in small, isolated coastal cetacean populations around the world. Inshore bottlenose dolphins were observed to leave the Clarence River, subtropical northern New South Wales, during periods of heavy rainfall and floods (Fury and Harrison 2011). Additionally, the link between floods, infectious diseases, accumulation of contaminants and mortality events is now well documented (Fury and Reif 2012, Meager and Limpus 2014). Land reclamation, vessel traffic, construction and dredging may result in the physical loss and degradation of habitat for cetaceans and affect the long-term viability of (Bejder et al. 2006, Jefferson et al. 2009, Pirotta et al. 2013, Pennino et al. 2016). In addition to their impacts on dolphin habitat, these activities may directly disturb cetaceans through physical displacement and increased underwater noise (Jensen et al. 2009, Pirotta et al. 2013, Rako et al. 2013). Noise pollution introduced into the marine environment by anthropogenic activities (e. dredging, pile-driving, underwater surveying, shipping) is known to have short-term detrimental effects on marine mammals by interfering with their ability to communicate, echolocate, and/or mask other important natural sounds (Tyack and Janik 2013, Wang et al. 2014). A study conducted on Irrawaddy dolphins suggested that Irrawaddy dolphins surfaced significantly less in the presence of motorized canoes (< 40 hp), speedboats (40–200 hp), and container barges (>1000 hp) (Kreb and Rahadi 2004).

Whilst, the combined effect of the flood and WBDDP are the likely causes of decline in the number of humpback dolphins observed in 2011 in Port Curtis, their effect was only temporary. Abundance estimates from the current study indicated that no variation in the number of humpback dolphins

was observed between 2014 and 2016 in absence of flood effects and during normal port operations. In this period groups of humpback dolphins were observed throughout the entire length of Port Curtis without showing apparent signs of stress.

Evidence of a significant decline is available for humpback dolphins in Port Alma. Following the initial major floods of the Fitzroy River during 2009, a small (~ 9%) non-significant decline was observed in this population (Cagnazzi 2013). The last available estimates for 2011 suggested that about 104 humpback dolphins were still living in the Keppel Bay region including Port Alma. Recent population estimates from the present study provide further evidence of ongoing decline and indicate that, between 2014 and 2016, the number of humpback dolphins in Port Alma declined significantly from an estimated size of 50–94 (2014–2015) to 29–42 (2016). During this period the capital dredging activities associated with the WBDDP had been completed and therefore cannot be suggested as a cause of the decline of humpback dolphins in Port Alma. However, the increased flood frequency experienced in Port Alma from 2010 to 2017 is likely to be a major factor in the observed decline. Inshore bottlenose dolphins were observed to leave the Clarence River, subtropical northern New South Wales, during periods of heavy rainfall and floods (Fury and Harrison 2011). Additionally, the link between floods, infectious diseases, accumulation of contaminants and mortality events is now well documented (Fury and Reif 2012, Meager and Limpus 2014).

Before 2009, the humpback dolphin was the most common species observed throughout the entire Fitzroy River system, with common sightings as far inland as the city of Rockhampton (40 km upstream from the river mouth) (Cagnazzi 2011). Since 2010, no humpback dolphin has been recorded in the main Fitzroy River system. Between 2007–2011 humpback dolphins were the most abundant species in Port Alma, however from 2011 onwards snubfin dolphins became the most abundant dolphins. The estimates of snubfin dolphins in Port Alma before 2011 (95% CI = 100–110) overlap with the estimates from this present study (95% CI = 85–142). It must be noted that the 2011 population estimates were obtained for a significantly larger survey area (~ 980 km^2) than Port Alma (~ 500 km^2) and used different survey designs and different population models (Jolly-Seber from 2007 to 2012 vs. Robust Design Model 2014 to 2016), and are therefore not directly comparable. In section 6.2 below, we propose a method to combine these available datasets to obtain comparable abundance estimates of humpback and snubfin dolphins from 2007 to 2016 in the ERMP survey area.

In addition to the significant decline observed in the three years of this study, analysis of genetic samples of humpback dolphins from Port Alma provided some evidence of a bottleneck, although this result was not supported by all mutation models and the overall statistical power of the analysis

was low. As top order predators, declines in dolphin abundance or their permanent movement to new habitats may signal degradation of the whole ecological system (Hawkins et al. 2017). The stable isotope analysis has shown that the fish prey in the diets of snubfin and humpback dolphins overlapped considerably. According to niche overlap theory, species that live in sympatry and have overlapping diets are expected to engage in interspecific competition unless there is some form of resource partitioning (Parra 2006, Spitz et al. 2006). Stable isotope analysis suggested that in Port Alma snubfin dolphins foraging preferences could be restricted to more inshore benthic habitats than those of humpback dolphins. As result of the decline in the number of humpback dolphins using Port Alma, snubfin dolphins may have experienced less interspecific competition and more food sources to support a larger population size in this region.

The high levels of OCs and heavy metals detected in the epidermidis and blubber samples from humpback and snubfin dolphins in the ERMP survey area is an additional cause for concern. The levels of PCBs, DDTs and HCB in biopsy samples collected between 2014 and 2016 were significantly higher than the levels of the same contaminants in samples collected from dolphins in the same area before 2010. Exposure to some level of contaminants in an area with high levels of industrial and agricultural activities is unavoidable. These contaminants may be also found at low concentrations in the natural environment, but can reach hazardous levels in top predator species like dolphins through the process of bioaccumulation. High levels of persistent organic pollutants and heavy metals are known to be associated with adverse health effects in marine mammals (Kannan et al. 2000, Jepson et al. 2005) including impairment of immune function (De Swart et al. 1996, Kannan et al. 2000), increased neonatal mortality (Schwacke et al. 2002), decreased in reproductive rates (Aguilar and Borrell 1994, Jepson et al. 2005) and are associated with carcinoma (Ylitalo et al. 2005). Subsequently, the link between large mortality events of marine mammals associated with major environmental impacts or disease outbreaks now has strong support in the scientific literature (Aguilar and Borrell 1994, Casalone et al. 2014, Kemper et al. 2016).

The ERMP survey area is already subjected to high levels of anthropogenic activities that will likely continue to increase in the future. The frequency of high flow events has increased in the GBR from 1 in every 20 yr prior to European settlement (1748–1847) to 1 in every 6 yr reoccurrence (1948–2011) (Lough et al. 2015). All atmospheric models forecast an increase in the frequency and intensity of cyclones, heavy rain and floods in the region (Abbs 2010). The risks of more large-scale dolphin mortality events are likely to reoccur, especially in dolphin populations already under stress from multiple cumulative impacts (Hawkins et al. 2017).

Overall there are significant concerns for humpback dolphins in Port Alma, considering the low potential biological removal (PBR = 2.21, 95%CI = 1.90–2.51) of this population (Cagnazzi et al.

2013c, Parra and Cagnazzi 2016). If the decline of humpback dolphins in Port Alma is confirmed from the analysis of the entire ten-year dataset (2007–2016) the long-term survival of this population may be at risk. At present, snubfin dolphins in Port Alma do not appear to face a similar risk. Similarly, the estimated number of humpback dolphins in Port Curtis has returned to the estimates prior to the WBDDP. However, since Port Alma was identified as a source population for humpback dolphins in Port Curtis, the decline in numbers of humpback dolphins in Port Alma may also affect the resilience of humpback dolphins in Port Curtis and their capacity of recover from future environmental changes.

6.2 Projects to conduct with currently available data

The large amount of data on dolphin populations collected during the present study from 2014 to 2016 using systematic survey protocols combined with previous data collected by Dr. Cagnazzi during extensive surveys from 2007 to 2013 will enable further investigations into the ecology and population dynamics of humpback and snubfin dolphins in the ERMP survey area, without the need for further fieldwork surveys.

More specifically, the following two investigations have important implications for the management and conservation of inshore dolphins in the ERMP survey area:

1. *Analysis of long-term mark-recapture data of humpback and snubfin dolphins in Port Alma and Port Curtis and development of population viability models including extinction risk under different scenarios parameterised using the genetic data and ten years of mark-recaptue data.*

   Between 2007 and 2013, dedicated boat-based surveys were conducted in Port Curtis and Port Alma. These surveys were conducted following standardised survey protocols compatible with those applied during surveys for this GPC/ERMP monitoring project (2014–2016). Therefore, with few adaptations, data collected during the two separate survey periods can be merged into a unique dataset and used to produce robust yearly estimates of humpback and snubfin dolphins in Port Alma and Port Curtis between 2007 and 2016. A Full-Capture Hierarchical Multistate Bayesian Model Based on the CRDM is recommended to be used for the analysis (Rankin et al. 2016). Based on the accuracy of the abundance estimates obtained during this study, and the 10 years of data, significant trends larger than 7% will be detected.

2. *Population viability analysis of humpback and snubfin dolphin in the ERMP survey area.*

The results from the mark-recapture analysis in association with demographic parameters derived for humpback and snubfin dolphins or similar species can be used to develop Population Viability Models (PVMs) for humpback and snubfin dolphin in the ERMP survey area. PVMs can be a valuable tool for investigating current and future risk of population decline under specific scenarios. Population viability can be analysed by building an age-structured, and where appropriate a spatially-structured, model of population dynamics of each of the two species. Parameter estimates for the demographic rates of these models will be based on the genetic and mark-recapture data that underpins other components of this project. However, the models will also draw on analyses of other dolphin species if required. The PVM could incorporate both demographic and environmental stochasticity in the population dynamics and can be used to predict times to extinction and risks of population decline (McCarthy and Possingham 2006) under different scenarios. Uncertainty in the parameters of the population models could be propagated via Bayesian analyses into uncertainty in the predictions of the model (McCarthy and Possingham 2007). This would clearly represent the uncertainty in the model's predictions given the data and other information sources that are available.

3. *Develop GIS-based spatially-explicit fine-scale models of humpback dolphins in Port Curtis and The Narrows of: (1) the distributions and relative abundance of humpback dolphins in the Port areas, (2) relevant threatening processes as a proxy for vulnerability and exposure to threats, and (3) use these maps to develop quantitative models of risk to the dolphins in the port area.*

   We propose using the large number of sightings collected between 2014-2016 using a standardised survey design based on stratified parallel transects to develop a fine scale species distribution model (SDM) for Port Curtis and The Narrows. We will also investigate the use of data collected prior to 2014 to develop species distribution model prior to the WBDDP and 2011 flood. SDMs require a minimum of 60 samples. A total of 130 sightings have been collected for Port Curtis alone in the three years of the study. This information combined with data on boat traffic and anthropogenic activities will allow identification of critical areas i.e. areas of high dolphin presence overlapping with elevated human activities. The results of the SDM can then help to define the boundaries of candidate spatial protection measures (e.g. the establishment of protected areas, or modification of potentially impacting activities such as shipping lanes) by providing a better description of the species' distribution compared to simpler measures of occurrence (Cañadas et al. 2005, Bailey and

Thompson 2009). These models aim to characterise a population's realised niche, i.e. the range of environmental conditions it occupies, resulting from the integration of "physiological performance and ecosystem constraints" (Guisan and Zimmermann 2000). Ultimately, these models would allow us to make robust predictions about a species' occurrence in space and time (Austin 2002, Elith and Leathwick 2009). We will use binary Generalized Additive Models (GAMs) to assess the relationship between the occurrence of dolphin groups and a series of environmental and temporal variables (Hastie and Tibshirani 1990, Wood 2006). Depending on the number of sightings and features of the data, it might be possible to investigate the spatio-temporal distribution of group sizes using Poisson GAMs, and generate density maps.

6.3 Recommendations for future studies

This project has provided abundance estimates with acceptable precision for humpback and snubfin dolphins in the ERMP survey area. The sampling design used to survey the ERMP survey area was developed to meet the requirements of a large-scale mark-recapture project and therefore, could not provide any information on fine scale movements patterns and population dynamics of humpback and snubfin dolphins in this region.

Brooks et al. (2017) developed a mark-recapture sampling protocol to monitor the movement patterns of inshore dolphins in Darwin Harbour, Northern Territory. Their survey design consisted of two primary periods per year (March/April and September/October), with nine secondary periods (days) in each primary period. The multi-state robust design model (MSCRD) was chosen to analyse the data as it offers the potential for assessing abundance estimates and distinguishes between movements to and from a site from demographic changes.

Such an intensive survey protocol cannot be applied to the entire ERMP survey area without having access to larger resources. Based on the results from this study, capture probabilities were only high enough in Port Curtis (at least 12 marked dolphins captured for each secondary period or six per day) to justify the application of MSCRD. Furthermore, Port Curtis is the only area within the ERMP survey area, where more development activities are currently planned for the future.

We therefore recommend to adapt the survey protocol used by Brooks et al. (2017) to monitor fine scale movements patterns and seasonal population dynamics of humpback dolphins in Port Curtis. We recommend increasing the number of primary periods to a minimum of four, approximately corresponding to the four seasons. Additional primary periods should be timed to monitor the effect of external environmental and anthropogenic factors (floods, cyclone, dredging, etc.) that may affect the normal movement patterns and seasonal population dynamics of humpback dolphins in

Port Curtis. This recommended project is essential to monitor seasonal changes and habitat use patterns of these dolphins.

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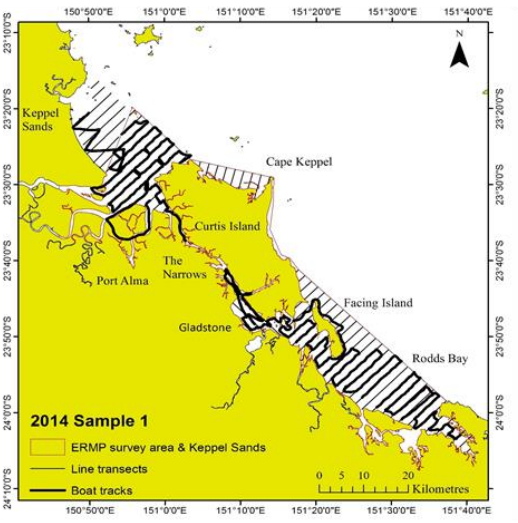
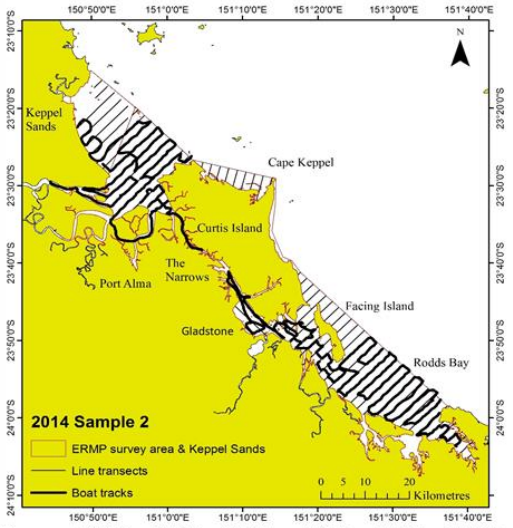
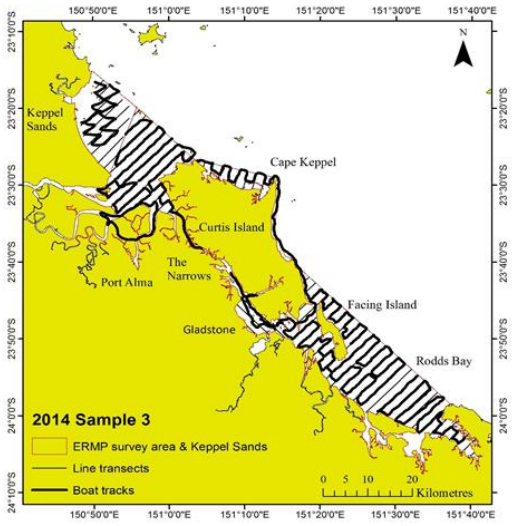
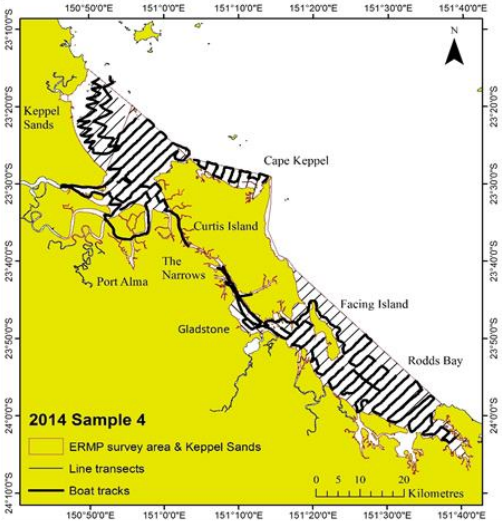
8 Appendices

8.1 Appendices Chapter 2.









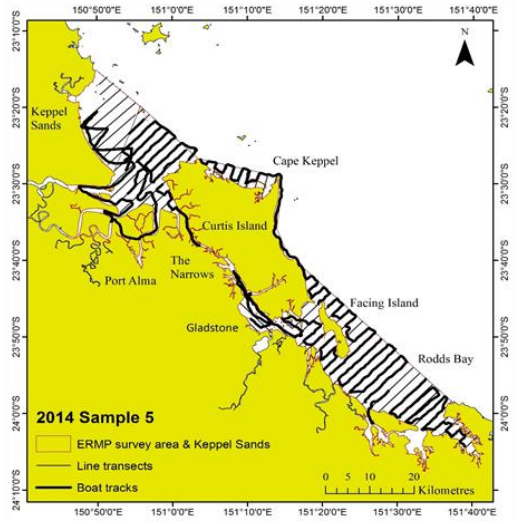
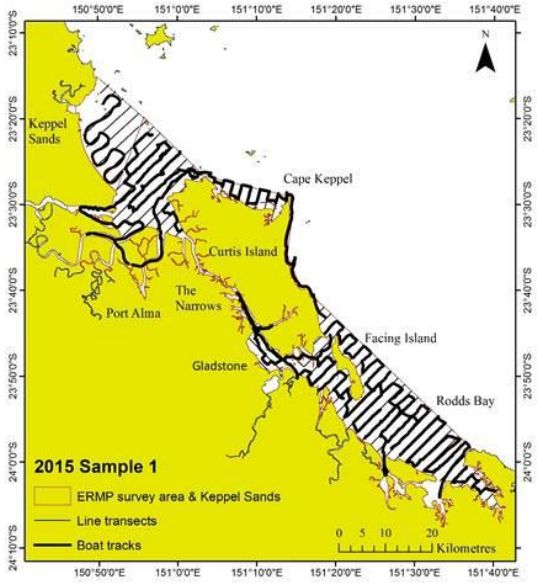
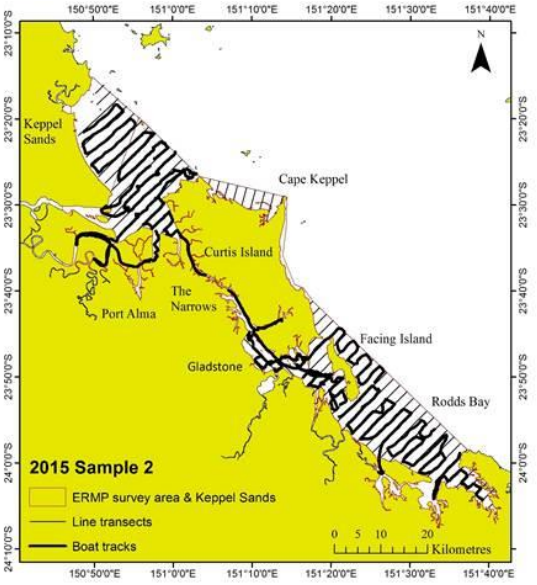
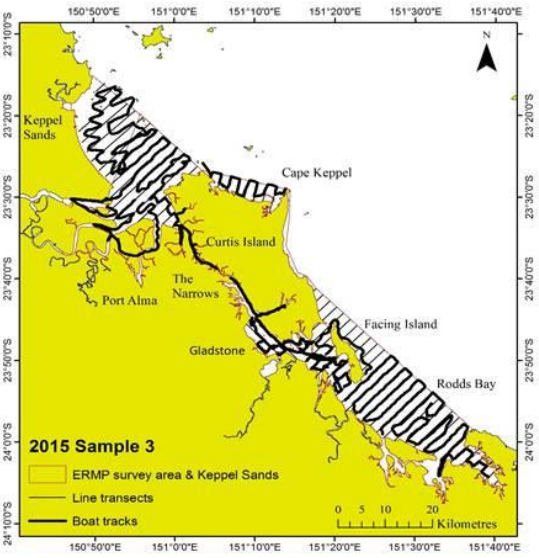
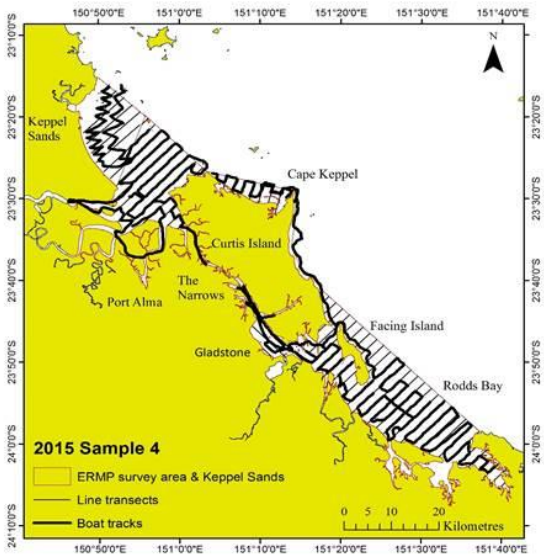


Figure A.1 The maps also show the tracks (transects) completed by the research vessels during boat-based line transects surveys conducted in 2014 in the ERMP survey area.









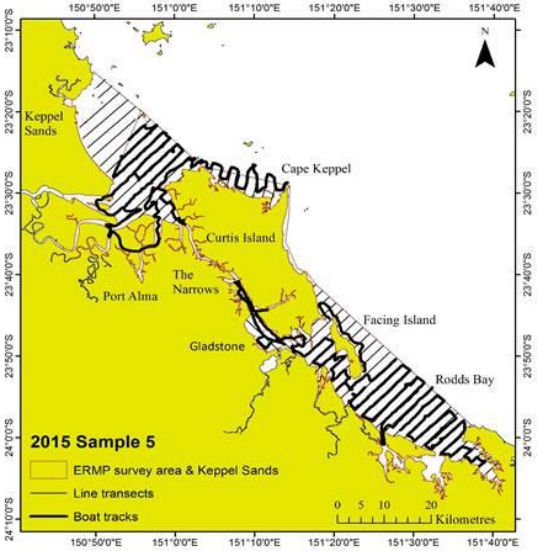


Figure A.2. The maps also show the tracks (transects) completed by the research vessels during boat-based line transects surveys conducted in 2015 in the ERMP survey area.

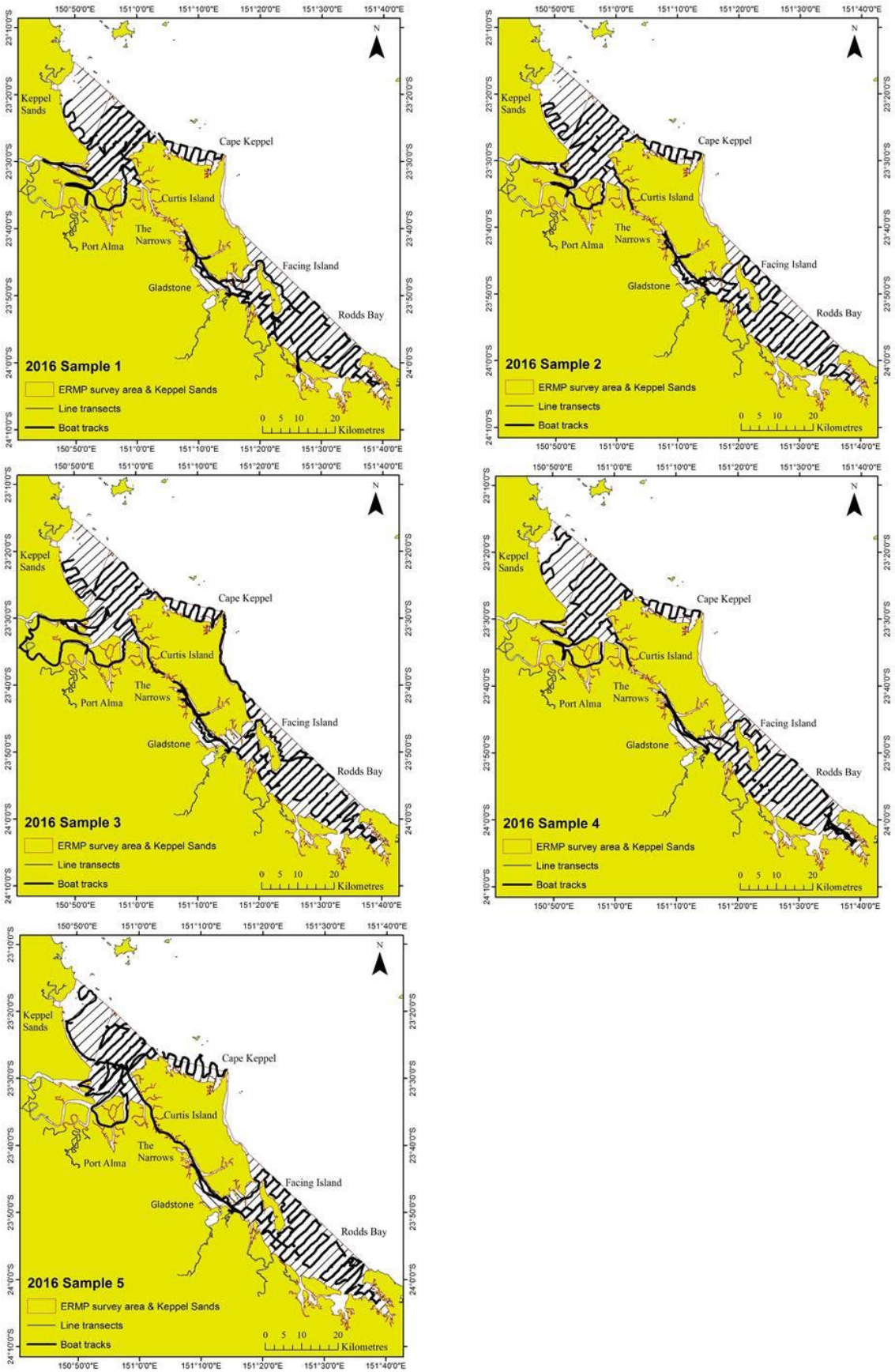


Figure A.3 The maps also show the tracks (transects) completed by the research vessels during boat-based line transects surveys conducted in 2015 in the ERMP survey area.

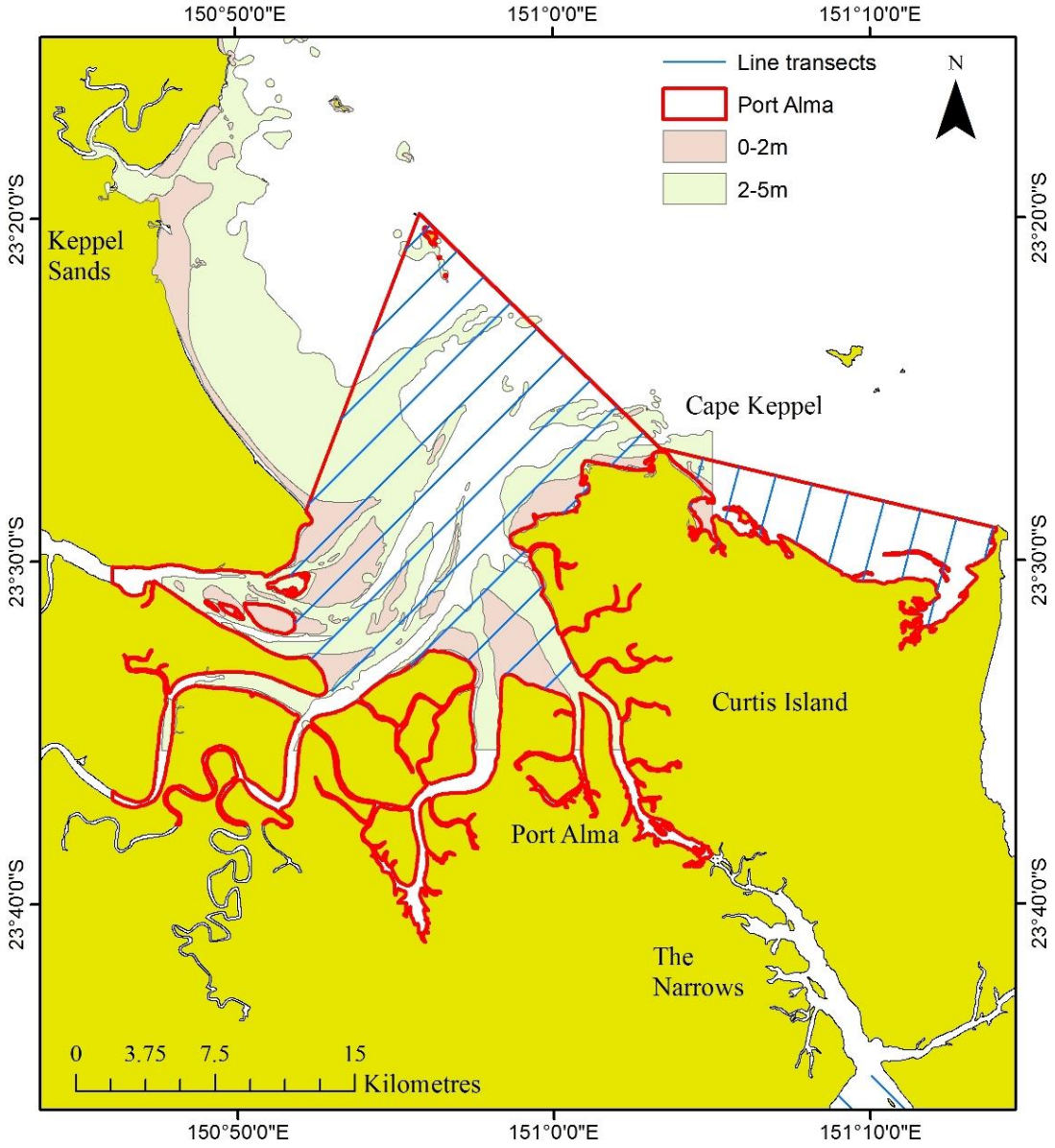


Figure A.4 Map of the Port Alma sub-area showing the proportion of transects inaccessible at low tide condition. Transect overlapping with water depth ranging from 0-2m are inaccessible at low tide

Table A.1 Model averaged parameter estimates for the best ranked Pollock's Closed Robust Design Model for snubfin dolphin using transect only photo-identification data in the ERMP survey area.

Parameters	Estimate	SE	LCI	UCI
Survival Parameter 1	0.68	0.14	0.37	0.89
Survival Parameter 2	0.54	0.17	0.24	0.81
Gamma	0.00	0.00	0.00	0.00
p Session 1	0.06	0.03	0.02	0.14
p Session 1	0.24	0.07	0.13	0.39
p Session 1	0.15	0.05	0.08	0.27
p Session 1	0.11	0.04	0.06	0.22
p Session 1	0.14	0.05	0.07	0.25
p Session 2	0.28	0.05	0.20	0.38
p Session 2	0.08	0.02	0.04	0.14
p Session 2	0.16	0.03	0.10	0.23
p Session 2	0.25	0.04	0.18	0.35
p Session 2	0.15	0.03	0.09	0.23
p Session 3	0.26	0.06	0.15	0.41
p Session 3	0.12	0.04	0.06	0.22
p Session 3	0.11	0.04	0.06	0.21
p Session 3	0.05	0.02	0.02	0.12
p Session 3	0.15	0.05	0.08	0.26
f0 Session 1	40.72	17.18	7.06	74.39
f0 Session 2	49.90	14.15	22.15	77.64
f0 Session 3	45.57	17.78	10.72	80.42

Table A.2 Model averaged parameter estimates for the best ranked Pollock's Closed Robust Design Model for snubfin dolphins using transect and biopsy sampling photo-identification data in the ERMP survey area.

Parameters	Estimate	SE	LCI	UCI
Survival Parameter 1	0.64	0.17	0.29	0.88
Survival Parameter 2	0.00	0.00	0.00	0.00
Gamma	0.12	0.05	0.06	0.25
p Session 1	0.19	0.04	0.12	0.28
p Session 2	0.12	0.04	0.06	0.22
p Session 3	42.57	33.21	-22.52	107.66
f0 Session 1	28.17	18.82	-8.71	65.05
f0 Session 2	39.72	29.27	-17.65	97.09
f0 Session 3	0.64	0.17	0.29	0.88

Table A.3 Model averaged parameter estimates for the best ranked Pollock's Closed Robust Design Model for humpback dolphins in the ERMP survey area.

Parameters	Estimate	SE	LCI	UCI
Apparent survival	0.71	0.05	0.61	0.80
Gamma	0.00	0.00	0.00	0.00
p Session 1	0.25	0.04	0.19	0.34
p Session 1	0.22	0.04	0.16	0.30
p Session 1	0.27	0.04	0.20	0.35
p Session 1	0.38	0.05	0.30	0.47
p Session 1	0.24	0.04	0.17	0.32
p Session 2	0.26	0.04	0.20	0.34
p Session 2	0.20	0.03	0.15	0.28
p Session 2	0.18	0.03	0.13	0.26
p Session 2	0.26	0.04	0.19	0.34
p Session 2	0.38	0.04	0.30	0.46
p Session 3	0.17	0.03	0.11	0.25
p Session 3	0.15	0.03	0.09	0.22
p Session 3	0.42	0.05	0.33	0.52
p Session 3	0.25	0.04	0.18	0.34
p Session 3	0.19	0.04	0.13	0.27
f0 Session 1	29.48	8.51	16.94	51.32
f0 Session 2	32.92	8.60	19.89	54.48
f0 Session 3	32.18	9.27	18.51	55.96

Table A.4 Model averaged parameter estimates for the best ranked Pollock's Closed Robust Design Model for humpback dolphins in Port Curtis and Rodds Bay dataset.

Parameters	Estimate	SE	LCI	UCI
Apparent survival	0.83	0.11	0.50	0.96
Gamma	0.00	0.00	0.00	0.00
p Session 1	0.36	0.12	0.17	0.61
p Session 1	0.24	0.10	0.10	0.49
p Session 1	0.28	0.11	0.12	0.53
p Session 1	0.36	0.12	0.17	0.61
p Session 1	0.31	0.11	0.14	0.56
p Session 2	0.23	0.09	0.10	0.45
p Session 2	0.18	0.08	0.07	0.39
p Session 2	0.21	0.09	0.08	0.42
p Session 2	0.21	0.09	0.08	0.42
p Session 2	0.39	0.11	0.20	0.61
p Session 3	0.18	0.09	0.06	0.42
p Session 3	0.11	0.07	0.03	0.34
p Session 3	0.52	0.13	0.28	0.75
p Session 3	0.24	0.10	0.10	0.48
p Session 3	0.18	0.09	0.06	0.42
f0 Session 1	14.09	12.18	-9.79	37.97
f0 Session 2	27.64	18.05	-7.73	63.01
f0 Session 3	21.39	16.35	-10.65	53.43

Table A.5 Model averaged parameter estimates for the best ranked in the ERMP survey model for humpback dolphins in Port Curtis dataset.

Parameters	Estimate	SE	LCI	UCI
Apparent survival	0.67	0.16	0.33	0.90
Gamma''	0.00	0.00	0.00	0.00
p Session 1	0.49	0.16	0.21	0.77
p Session 1	0.20	0.12	0.05	0.52
p Session 1	0.26	0.13	0.08	0.57
p Session 1	0.49	0.16	0.21	0.77
p Session 1	0.33	0.14	0.12	0.64
p Session 2	0.26	0.13	0.09	0.56
p Session 2	0.15	0.10	0.04	0.46
p Session 2	0.18	0.11	0.05	0.48
p Session 2	0.19	0.11	0.05	0.50
p Session 2	0.46	0.15	0.21	0.74
p Session 3	0.26	0.15	0.07	0.61
p Session 3	0.16	0.12	0.03	0.52
p Session 3	0.54	0.19	0.21	0.85
p Session 3	0.08	0.09	0.01	0.46
p Session 3	0.10	0.09	0.01	0.47
f0 Session 1	6.89	8.76	-10.27	24.05
f0 Session 2	17.13	15.58	-13.42	47.67
f0 Session 3	14.38	15.87	-16.72	45.48

Table A.6 Model averaged parameter estimates for the best ranked Pollock's Closed Robust Design Model for humpback dolphins in Port Alma dataset.

Parameters	Estimate	SE	LCI	UCI
Apparent survival	0.45	0.08	0.30	0.61
Gamma	0.00	0.00	0.00	0.00
p Session 1	0.06	0.03	0.02	0.16
p Session 1	0.16	0.05	0.08	0.29
p Session 1	0.22	0.06	0.12	0.37
p Session 1	0.35	0.08	0.21	0.52
p Session 1	0.11	0.04	0.05	0.23
p Session 2	0.40	0.09	0.24	0.58
p Session 2	0.31	0.08	0.17	0.49
p Session 2	0.12	0.06	0.05	0.29
p Session 2	0.34	0.09	0.19	0.52
p Session 2	0.37	0.09	0.22	0.55
p Session 3	0.12	0.06	0.04	0.30
p Session 3	0.25	0.09	0.12	0.45
p Session 3	0.06	0.04	0.01	0.22
p Session 3	0.25	0.09	0.12	0.45
p Session 3	0.19	0.08	0.08	0.38
f0 Session 1	22.04	10.24	9.27	52.41
f0 Session 2	4.49	3.10	1.32	15.25
f0 Session 3	12.34	7.13	4.31	35.33

8.2 Appendices Chapter 3

Table A.7 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
21288	81.2	1.85	2.53	22291	19.99	1.77	1.87
21289	81.9	1.65	2.38	22292	47.51	1.85	2.37
21290	36.03	1.38	2.23	22293	156.84	1.69	1.74
21291	88.92	2.74	2.30	22294	0.98	1.12	0.11
21292	07.52	1.57	2.88	22295	181.32	1.85	2.35
				22296	47.3	1.68	1.59
				22297	81.32	1.58	2.53
					81.29	1.9	2.83
					336.03	1.83	2.22
					288.29	1.74	2.03
					107.52	1.75	1.88

Table A.8 Summary analysis for presence of null allele and significant deviation from HWE in samples of humpback dolphins collected from a) Port Alma, b) Port Curtis, c) Whitsundays, d) Northern Great Sandy Strait and e) Southern Great Sandy Strait. Analyses have been conducted with Microchecker (Van Oosterhout et al. 2004a). Results are presented based on the four methods (Van Oosterhout, Chakraborty and Brookfield) of null allele estimation available in Microchecker (Chakraborty et al. 1992, Brookfield 1996, Van Oosterhout et al. 2004b).

a) Port Alma

Locus	Null Present	Van Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
KWM12	no	-0.1889	-0.0935	-0.0456	0
MK6	no	-0.067	-0.0256	-0.0058	0
MK8	no	-0.0111	-0.0095	-0.0084	0
MK9	yes	0.136	0.2483	0.0713	0.0713
TUR4_117	no	0.0074	0.0075	0.0039	0.0039
TUR4_128	no	-0.0023	0.0049	0.0029	0.0029
TUR4_138	no	-0.067	-0.0256	-0.0058	0
TUR4_141	no	0.143	0.1778	0.0958	0.1766
TUR4_142	no	-0.0933	-0.0802	-0.0672	0
TUR4_153	no	-0.0112	-0.0088	-0.0069	0
TUR4_162	no	-0.009	-0.0088	-0.0057	0
TUR4_66	no	0.053	0.0519	0.0314	0.0314
TUR4_80	no	0.0256	0.0267	0.0167	0.0167
TUR4_91	no	-0.1262	-0.0518	-0.0196	0.3185

One locus shows evidence for a null allele.
This population is possibly in Hardy Weinberg equilibrium with locus MK9, showing signs of a null allele.

b) Port Curtis

Locus	Null Present	Van Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
KWM12	no	0.0413	0.0352	0.0214	0.0214
MK6	no	-0.1762	-0.0874	-0.0407	0
MK8	no	0.0953	0.145	0.0407	0.0407
MK9	no	0.0227	0.0283	0.023	0.023
TUR4_117	no	0.0197	0.0168	0.0095	0.0095
TUR4_128	no	-0.036	-0.0136	-0.0018	0
TUR4_138	no	-0.0263	-0.0467	-0.0291	0
TUR4_141	no	-0.0364	-0.0182	-0.0024	0
TUR4_142	no	-0.0158	-0.0381	-0.0198	0.2655
TUR4_153	no	-0.0037	-0.009	-0.0063	0
TUR4_162	no	0.0557	0.0604	0.0429	0.0429
TUR4_66	no	-0.2681	-0.1313	-0.0795	0
TUR4_80	no	0.0299	0.0313	0.0201	0.0201
TUR4_91	no	-0.0244	-0.0235	-0.0155	0

No loci show evidence for a null allele.
This population is probably in Hardy Weinberg equilibrium.

c) Whitsundays

Locus	Null Present	Van Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
KWM12	no	-0.2679	-0.1034	-0.0667	0
MK6	no	0	0	0	0
MK8	no	-0.1504	-0.1256	-0.1163	0
MK9	no	0.1511	0.1795	0.1273	0.1273
TUR4_117	no	-0.0039	0.0078	0.0052	0.0052
TUR4_128	no	-0.0987	-0.0458	-0.0348	0
TUR4_138	no	-0.2929	-0.1429	-0.0909	0
TUR4_141	no	-0.0646	-0.0323	-0.007	0
TUR4_142	no	0.2196	0.4182	0.1377	0.1377
TUR4_153	no	0.0153	0.0184	0.0142	0.0142
TUR4_162	no	-0.327	-0.2133	-0.2133	0
TUR4_66	no	0.2906	0.5493	0.2131	0.2131
TUR4_80	no	0.1441	0.2	0.0909	0.0909
TUR4_91	no	0.0607	0.068	0.0383	0.0383

No loci show evidence for a null allele.
This population is probably in Hardy Weinberg equilibrium.

d) Northern Great Sandy Strait

Locus	Null Present	Van Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
KWM12	no	-0.1502	-0.0746	-0.0311	0
MK6	no	-0.5918	-0.2632	-0.2336	0
MK8	no	-0.0572	-0.0286	-0.0056	0
MK9	no	0.1486	0.28	0.0722	0.0722
TUR4_117	no	-0.1783	-0.0773	-0.0372	0
TUR4_128	no	-0.0282	-0.0141	-0.0015	0
TUR4_138	no	-0.1835	-0.0909	-0.0435	0
TUR4_141	no	0	0	0	0
TUR4_142	no	-0.1502	-0.0746	-0.0311	0
TUR4_153	no	-0.1203	-0.1015	-0.0752	0
TUR4_162	no	0.0869	0.1093	0.0578	0.0578
TUR4_66	no	0.0522	0.0601	0.027	0.027
TUR4_80	no	-0.0893	-0.0778	-0.0544	0
TUR4_91	no	0.0787	0.0924	0.0485	0.0485

No loci show evidence for a null allele.
This population is probably in Hardy Weinberg equilibrium.

e) Southern Great Sandy Strait

Locus	Null Present	Van Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
KWM12	no	-0.1297	-0.0511	-0.0198	0
MK6	no	-0.2362	-0.1163	-0.0653	0
MK8	no	-0.2168	-0.0762	-0.0435	0
MK9	no	0.0803	0.0649	0.0442	0.0442
TUR4_117	no	-0.0468	-0.0323	-0.0213	0
TUR4_128	no	-0.134	-0.0667	-0.0256	0
TUR4_138	no	-0.2113	-0.1566	-0.1215	0
TUR4_141	no	-0.134	-0.0667	-0.0256	0
TUR4_142	no	0.0626	0.0813	0.0424	0.0424
TUR4_153	no	-0.0918	-0.0804	-0.058	0
TUR4_162	no	0.0423	0.0035	0.0023	0.0023
TUR4_66	no	0.1052	0.1377	0.0601	0.0601
TUR4_80	no	-0.134	-0.1089	-0.078	0
TUR4_91	no	-0.4226	-0.2	-0.1538	0

No loci show evidence for a null allele.
This population is probably in Hardy Weinberg equilibrium.

Table A.9 Summary analysis of linkage disequilibrium for each locus across all populations for samples of humpback dolphins. Analyses have been conducted with Genepop (Rousset 2008).

Locus PAIR	χ^2	df	*p*-value	Locus PAIR	χ^2	df	*p*-value
E12 & KWM12	5.052	8	0.751	KWM12 & TUR153	2.528	8	0.960
E12 & MK6	13.805	10	0.182	MK6 & TUR153	26.948	10	0.002
KWM12 & MK6	0	8	1	MK8 & TUR153	7.900	10	0.638
E12 & MK8	11.002	10	0.357	MK9 & TUR153	17.738	10	0.059
KWM12 & MK8	6.046	8	0.642	TUR117 & TUR153	8.899	10	0.541
MK6 & MK8	8.851	10	0.546	TUR128 & TUR153	10.886	10	0.366
E12 & MK9	14.462	10	0.152	TUR138 & TUR153	10.797	8	0.213
KWM12 & MK9	8.982	8	0.343	TUR141 & TUR153	16.420	10	0.088
MK6 & MK9	14.214	10	0.163	TUR142 & TUR153	14.284	10	0.160
MK8 & MK9	15.176	10	0.125	E12 & TUR162	9.430	10	0.491
E12 & TUR117	8.732	10	0.557	KWM12 & TUR162	8.259	8	0.408
KWM12 & TUR117	9.819	8	0.277	MK6 & TUR162	9.671	10	0.469
MK6 & TUR117	13.817	10	0.181	MK8 & TUR162	8.482	10	0.581
MK8 & TUR117	15.809	10	0.105	MK9 & TUR162	16.680	10	0.081
MK9 & TUR117	12.208	10	0.271	TUR117 & TUR162	5.892	10	0.824
E12 & TUR128	4.586	10	0.917	TUR128 & TUR162	6.910	10	0.733
KWM12 & TUR128	1.679	8	0.342	TUR138 & TUR162	7.9416	8	0.439
MK6 & TUR128	18.932	10	0.041	TUR141 & TUR162	6.431	10	0.777
MK8 & TUR128	8.032	10	0.625	TUR142 & TUR162	14.388	10	0.156
MK9 & TUR128	6.410	10	0.779	TUR153 & TUR162	5.3245	10	0.868
TUR117 &TUR128	12.474	10	0.254	E12 & TUR66	11.279	10	0.336
E12 & TUR138	10.364	8	0.240	KWM12 & TUR66	6.333	8	0.609
KWM12 & TUR138	0	6	1	MK6 & TUR66	9.282	10	0.505
MK6 & TUR138	7.048	8	0.531	MK8 & TUR66	6.440	10	0.777
MK8 & TUR138	7.020	8	0.534	MK9 & TUR66	13.093	10	0.218

Table A.10 Summary analysis of linkage disequilibrium for each locus across all populations for samples of humpback dolphins. Analyses have been conducted with Genepop (Rousset 2008).

Locus PAIR	χ^2	df	*p*-value	Locus PAIR	χ^2	df	*p*-value
MK9 & TUR138	8.967	8	0.345	TUR117 & TUR66	7.751	10	0.653
TUR117 & TUR138	24.25	8	0.002	TUR128 & TUR66	13.955	10	0.175
TUR128 & TUR138	9.190	8	0.326	TUR138 & TUR66	5.569	8	0.695
E12 & TUR141	8.525	10	0.577	TUR141 & TUR66	15.248	10	0.123
KWM12 & TUR141	1.522	8	0.992	TUR142 & TUR66	13.527	10	0.195
MK6 & TUR141	15.02	10	0.131	TUR153 & TUR66	6.874	10	0.737
MK8 & TUR141	17.802	10	0.058	TUR162 & TUR66	13.410	10	0.201
MK9 & TUR141	3.539	10	0.965	E12 & TUR80	6.676	10	0.755
TUR117 & TUR141	4.842	10	0.901	KWM12 & TUR80	1.639	8	0.990
TUR128 & TUR141	8.743	10	0.556	MK6 & TUR80	8.443	10	0.585
TUR138 & TUR141	5.054	8	0.751	MK8 & TUR80	21.695	10	0.016
E12 & TUR142	11.234	10	0.339	MK9 & TUR80	7.148	10	0.711
KWM12 & TUR142	10.594	8	0.225	TUR117 & TUR80	5.552	10	0.851
MK6 & TUR142	7.077	10	0.718	TUR128 & TUR80	13.007	10	0.223
MK8 & TUR142	18.161	10	0.052	TUR138 & TUR80	0.895	8	0.998
MK9 & TUR142	8.994	10	0.532	TUR141 & TUR80	23.483	10	0.009
TUR117 & TUR142	5.668	10	0.842	TUR142 & TUR80	13.852	10	0.179
TUR128 & TUR142	16.485	10	0.086	TUR153 & TUR80	13.218	10	0.211
TUR138 & TUR142	2.8282	8	0.944	TUR162 & TUR80	12.386	10	0.260
TUR141 & TUR142	8.575	10	0.572	TUR66 & TUR80	3.403	10	0.970
E12 & TUR153	12.892	10	0.229				

Table A.11 Summary analysis for presence of null allele and significant deviation from HWE in samples of snubfin dolphins collected from Port Alma and Whitsundays. Analyses have been conducted with Microchecker (Van Oosterhout et al. 2004a). Results are presented based on the four methods (Van Oosterhout, Chakraborty and Brookfield) of null allele estimation available in Microchecker (Chakraborty et al. 1992, Brookfield 1996, Van Oosterhout et al. 2004b).

Locus	Null Present	Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
E12	no	0.0778	0.084	0.0558	0.0558
KWM12	no	-0.1548	-0.0769	-0.0328	0
MK3	no	-0.123	-0.0474	-0.0177	0
TUR4_105	no	0.1276	0.1504	0.089	0.089
TUR4_117	no	0.0989	0.0769	0.0476	0.0476
TUR4_142	no	0.1182	0.1504	0.089	0.089
TUR4_153	no	0.0047	-0.0153	-0.0127	0
TUR4_80	no	-0.0792	-0.0608	-0.0521	0
TUR4_87	no	0.1216	0.1391	0.0747	0.0747

No loci show evidence for a null allele.
This population is probably in Hardy Weinberg equilibrium.

Table A.12 Summary analysis of linkage disequilibrium for each locus across all populations for samples of snubfin dolphins. Analyses have been conducted with Genepop (Rousset 2008).

Locus PAIR	Chi2	df	*p*-value	Locus PAIR	Chi2	df	*p*-value
E12 & KWM12	6.992	4	0.136	TUR105 & TUR153	2.520	4	0.640
E12 & MK3	10.089	4	0.038	TUR117 & TUR153	6.758	4	0.149
KWM12 & MK3	6.083	4	0.193	TUR142 & TUR153	6.662	4	0.154
E12 & TUR105	2.574	4	0.631	E12 & TUR80	5.035	4	0.283
KWM12 & TUR105	19.225	4	0.000	KWM12 & TUR80	5.170	4	0.270
MK3 & TUR105	6.111	4	0.190	MK3 & TUR80	2.613	4	0.624
E12 & TUR117	4.679	4	0.321	TUR105 & TUR80	3.637	4	0.457
KWM12 & TUR117	2.132	4	0.711	TUR117 & TUR80	8.464	4	0.075
MK3 & TUR117	6.046	4	0.195	TUR142 & TUR80	4.514	4	0.340
TUR105 &TUR117	1.301	4	0.861	TUR153 & TUR80	3.445	4	0.486
E12 & TUR142	7.171	4	0.127	E12 & TUR87	1.176	4	0.88
KWM12 & TUR142	2.401	4	0.662	KWM12 & TUR87	4.504	4	0.342
MK3 & TUR142	0.706	4	0.950	MK3 & TUR87	3.992	4	0.407
TUR105 & TUR142	3.526	4	0.473	TUR105 & TUR87	4.445	4	0.349
TUR117 & TUR142	2.497	4	0.645	TUR117 & TUR87	4.027	4	0.402
E12 & TUR153	2.020	4	0.732	TUR142 & TUR87	1.881	4	0.757
KWM12 & TUR153	3.738	4	0.442	TUR153 & TUR87	0.267	4	0.991
MK3 & TUR153	5.758	4	0.217	TUR80 & TUR87	8.665	4	0.070

8.3 Appendices chapter 4

Table A.13 Descriptive statistics for 30 PCBs congeners, DDTs and HCB in biopsy samples of humpback and snubfin dolphins, summarised with mean and range (minimum and maximum) values. Values are expressed in ng/g of lipid weight (lw).

Species	**Humpback dolphins**				**Snubfin dolphins**			
Congeners	**Mean**	**Max**	**Min**	**SE**	**Mean**	**Max**	**Min**	**SE**
95	933.92	1545.67	46.38	97.23	616.91	1473.15	205.18	73.29
101	792.17	1743.87	39.99	93.35	487.94	1178.28	107.08	58.56
99	214.94	426.86	9.62	25.76	219.89	1079.69	0.00	59.25
151	496.75	927.02	19.24	65.93	453.74	1024.22	141.19	49.80
144+135	410.85	757.87	13.54	51.03	401.30	766.03	158.34	39.77
149+118	2090.77	3767.21	75.83	260.58	1919.33	4002.77	795.44	194.49
146	584.00	1367.92	14.35	102.36	587.05	1431.19	221.20	74.42
153	3919.47	9233.13	109.57	671.17	4181.00	9762.87	1577.9	504.98
141	12283.3	192576	11.32	11274	1114.73	10575.9	45.48	578.93
138	1854.29	4516.29	49.61	318.56	1853.67	4415.15	717.31	225.65
178	281.68	867.10	9.02	46.41	236.62	544.08	89.00	28.54
187	956.16	2461.78	23.60	179.41	1021.05	2473.29	309.83	131.40
183	297.45	795.48	5.68	53.37	290.81	738.68	79.66	37.18
128	159.69	494.69	3.61	29.66	162.97	397.55	43.50	20.82
174	311.94	698.72	7.68	45.01	286.48	663.91	101.51	36.70
177	219.86	476.09	5.24	32.11	195.36	473.13	62.86	24.79
156+171+202	178.28	403.59	5.20	24.00	118.06	262.79	48.72	13.15
172	137.76	364.36	0.00	23.45	80.35	198.84	0.00	10.80
180	909.07	2852.67	13.60	202.52	846.04	2064.50	198.96	122.39
199	211.03	919.35	0.00	55.04	223.25	674.68	0.00	47.92
170	564.31	1601.22	9.36	116.78	411.04	1074.36	175.36	58.93
196	171.91	507.55	0.00	33.04	110.50	268.44	0.00	18.16
201	373.59	2255.34	0.00	130.90	175.78	486.27	50.85	30.80
195	236.02	2053.72	0.00	124.04	97.50	564.73	0.00	39.72
194	128.51	505.37	0.00	29.86	107.93	282.45	23.53	16.54
206	35.38	297.91	0.00	19.36	17.43	227.73	0.00	12.81
op'DDE	966.87	2419.80	51.26	147.58	434.37	1485.17	91.85	80.02
pp'DDE	29911.6	64311.98	1318.4	3346.3	19907.6	43916.0	5710.19	2195.98
op'DDD	922.95	2213.09	49.44	146.04	381.93	1305.35	103.22	74.25
ppDDD	253.42	642.06	9.74	36.17	160.49	511.69	58.41	24.75
op'DDT	886.25	1601.17	40.86	92.51	558.25	1233.40	174.70	68.94
pp'DDT	1598.99	3374.73	82.80	237.41	724.97	2323.75	197.38	129.58
HCB	152.86	402.41	5.68	25.47	72.30	293.47	22.71	15.27

Table A.14 Descriptive statistics for 30 PCBs congeners, DDTs and HCB in biopsy samples of humpback and snubfin dolphins, summarised with mean and range (minimum and maximum) values. Values are expressed in ng/g of wet weight.

Species	**Humpback dolphins**				**Snubfin dolphins**			
Congeners	**Mean**	**Max**	**Min**	**SE**	**Mean**	**Max**	**Min**	**SE**
HCB	34.13	23.98	89.53	5.82	12.47	40.35	4.16	2.011229
95	202.88	117.83	519.04	28.58	111.14	202.56	37.61	11.09601
op'DDE	213.08	140.98	578.07	34.19	76.56	204.21	16.84	11.1942
101	170.90	90.92	389.24	22.05	88.04	162.01	19.63	8.827667
99	44.18	22.75	106.29	5.52	38.20	188.62	0.00	9.89305
pp'DDE	6305.60	3244.45	14199.34	786.90	3590.56	6268.29	1046.68	335.9728
op'DDD	203.32	148.08	634.31	35.91	66.68	179.49	18.41	10.3956
151	98.65	57.95	288.86	14.06	83.05	174.02	25.88	9.030477
144+135	83.36	47.85	227.12	11.60	74.08	130.15	29.02	7.223896
149+118	419.12	238.23	1173.86	57.78	349.84	680.07	145.80	33.82523
pp'DDD	52.93	32.61	135.15	7.91	28.15	70.36	10.71	3.262564
op'DDT	186.80	92.55	419.61	22.45	99.10	209.55	32.02	10.27179
146	112.32	82.08	356.92	19.91	107.13	243.16	35.25	13.22664
153	754.74	545.94	2463.75	132.41	759.02	1658.71	242.53	84.55764
141	2640.40	9778.70	40537.27	2371.68	191.90	1682.63	8.34	92.90692
pp'DDT	345.19	224.81	919.17	54.52	125.25	319.52	42.16	17.73063
138	355.10	243.07	1037.27	58.95	336.68	750.13	110.25	38.16275
178	56.95	39.15	182.53	9.50	42.79	92.44	13.68	4.797359
187	181.69	138.74	562.21	33.65	186.02	420.21	47.62	22.3557
183	57.30	40.97	159.74	9.94	53.28	125.50	12.24	6.521616
128	31.00	22.17	89.08	5.38	29.54	67.54	6.69	3.557513
174	61.14	35.35	147.64	8.57	51.92	112.80	15.60	6.364467
177	43.63	28.34	110.34	6.87	35.32	80.38	9.66	4.265964
156+171+202	37.79	26.93	107.39	6.53	21.28	44.65	8.17	2.175495
172	30.15	22.89	76.70	5.55	14.57	28.41	0.00	1.671621
180	172.26	155.48	600.49	37.71	156.07	350.76	30.58	21.76158
199	43.99	39.72	122.55	9.63	37.87	101.60	0.00	7.150366
170	106.62	88.06	337.06	21.36	76.37	200.46	28.92	11.48219
196	38.23	30.53	89.47	7.41	20.94	60.77	0.00	3.658064
201	72.38	91.70	359.95	22.24	30.51	71.28	11.04	4.450161
195	50.43	107.72	431.90	26.13	15.74	80.70	0.00	5.955312
194	27.00	28.24	106.38	6.85	19.45	41.56	5.15	2.844472
206	6.94	14.35	40.49	3.48	2.80	31.31	0.00	1.836086