



## Relative abundance, population genetic structure and passive acoustic monitoring of Australian snubfin and humpback dolphins in regions within the Kimberley

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## WAMSI Kimberley Marine Research Program Final Report

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## **WAMSI Kimberley Marine Research Program**

Initiated with the support of the State Government as part of the Kimberley Science and Conservation Strategy, the Kimberley Marine Research Program is co-invested by the Western Australian Marine Science Institution (WAMSI) partners to provide regional understanding and baseline knowledge about the Kimberley marine environment. The program has been created in response to the extraordinary, unspoilt wilderness value of the Kimberley and increasing pressure for development in this region. The purpose is to provide science based information to support decision making in relation to the Kimberley marine park network, other conservation activities and future development proposals.

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Image 1: Satellite image of the Kimberley coastline

Image 2: Snubfin dolphin (Image: Felix Smith)

Image 3: Humpback whale breaching, Exmouth (Image: Pam Osborn)

Image 4: Researchers retrieving an acoustic logger in Cygnet Bay (Image: Alex Brown).

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## **Executive summary**

The Australian snubfin dolphin (*Orcaella heinsohni*, 'snubfin dolphin' hereafter) and Australian humpback dolphin (*Sousa sahulensis*, 'humpback dolphin' hereafter) are poorly-understood species of dolphin whose global distribution is restricted to shallow coastal and estuarine waters of northern Australia and southern New Guinea.

Here, we investigate the population genetic structure and relative abundance of these two species at selected study sites in the Kimberley region of north-western Australia. Additionally, we investigate the application of passive acoustic monitoring (PAM) as an effective technique for monitoring these species in the remote waters of the Kimberley region, with potential applications across northern Australia.

## **Summary of Results**

### *Genetic analyses*

Genetic analyses expanded the geographic scope and sample sizes from previous assessments of population genetic structure of both snubfin and humpback dolphins. Further evidence of limited gene flow between snubfin dolphins at Cygnet Bay and Roebuck Bay (c. 250km distant) was revealed, whereas no significant differentiation was observed between snubfin dolphins at Cygnet and Cone Bay (c. 60km distant). Limited numbers of humpback dolphin samples were available, although comparison of samples pooled from all Kimberley sites with existing data from sites in the Pilbara revealed very little gene flow between the two regions.

### *Relative abundance*

Boat-based visual surveys and photo-identification revealed the presence of snubfin and humpback dolphins at all surveyed sites, albeit in variable numbers and degrees of approachability by boat. Snubfin dolphin encounter rates were lower ( $\leq 0.20$  dolphins/km effort) than for previously surveyed sites around the Dampier Peninsula, although the identification of 27 distinctively-marked individuals at Cone Bay, many of which were observed in both 2014 and 2015, suggests that this area is regularly used by a small number of snubfin dolphins. The Prince Regent River also appeared to support a small aggregation of snubfin dolphins. At each surveyed site, the relative abundance of humpback dolphins was fairly low ( $\leq 0.15$  dolphins/km effort) and comparable to those previously surveyed around the Dampier Peninsula. Despite low encounter rates from stratified surveys, groups of up to 11 humpback dolphins were regularly observed in the vicinity of the fish farm within Cone Bay. Difficulties in approaching dolphins by boat limited the effectiveness of photo-identification techniques at some sites, most notably the Cambridge Gulf in the eastern Kimberley, which receives the least vessel traffic.

### *Passive Acoustic Monitoring*

PAM was conducted at three sites within the Kimberley during 2014 and 2015 using *a priori* information of dolphin visual survey data. The soundscape of both Cygnet Bay and Roebuck Bay predominantly consisted of biological noise and low levels of man-made noise with little overlap of dolphin sounds, making them ideal sites to test PAM. Our investigation into the feasibility of using PAM for humpback and snubfin dolphins has furthered our understanding of the acoustic repertoire of these species, for which there has been limited knowledge for either species (particularly snubfin dolphins), which is a fundamental component of PAM.

In Roebuck Bay, dolphins were detected acoustically in approximately 66% of boat-based surveys that detected dolphins visually. The behaviour of the dolphins varied among the different sites which affected their vocalisation rates; socialising behaviour elicited the highest vocal rate, although was not always the most prominent behaviour within their activity budget. Consequently, when using PAM to assess the occurrence and density of snubfin and humpback dolphins, careful consideration needs to be given to their behaviour, the duration of time they spend in an area, and their typical group sizes; *a priori* information on these is desirable.

Currently, the application of PAM to snubfin and humpback dolphins is limited to monitoring occurrence. Further applications of PAM to these species will require the development of more efficient automatic detection algorithms for processing large acoustic datasets, developments in absolute abundance estimation

methods from acoustic data, and further progress towards resolving reliable specific identification parameters of their vocalisations.

## Implications for management

Our results provide managers and policy-makers with valuable data on the relative importance of several sites within the Kimberley region to snubfin and humpback dolphins, along with an improved understanding of the connectivity of populations and the appropriate geographic scales at which to manage them.

Our results indicate: (1) evidence of genetically distinct humpback dolphin populations in the Pilbara and Kimberley regions; (2) evidence of low gene flow between snubfin dolphins in Roebuck Bay and areas in King Sound; and, (3) limited evidence of a possible third snubfin dolphin genetic population north/east of King Sound. Our collaborative approach to boat-based surveys, which included training and participation by Traditional Owners and a Marine Park Joint Management team, has resulted in an improved understanding and capacity of relevant local land and sea managers to collect data on these poorly-understood species within remote areas, and laid the foundation for ongoing research in the Kimberley region.

Our investigation into the feasibility of using PAM has shown that this method effectively detects dolphins in high-use areas. Moreover, our study on its application for monitoring snubfin and humpback dolphins has advanced our limited understanding of the species' acoustic repertoire – a critical component of developing species-specific PAM tools. Managers of marine areas are constantly looking for cost-efficient methods for monitoring marine fauna. PAM is typically less expensive than other survey methods (i.e. aerial or boat-based surveys), and is becoming increasingly effective and accessible with continuous improvements in acoustic technology and decreasing costs of acoustic receivers. PAM is a very active area of research in relation to statistical analyses for abundance estimation, survey design, acoustic logging technologies and data processing algorithms. Although PAM is currently limited to monitoring the occurrence of snubfin and humpback dolphins, these results have advanced our ability to develop PAM as a more flexible tool for cost-effective monitoring.

Currently PAM can be used to:

- Confirm the presence of dolphins (but it cannot rule out the absence);
- Spatially monitor the occurrence of acoustically active dolphins using a grid of receivers (which can represent a large proportion of animals using an area);
- Temporally monitor occurrence and distribution cost effectively over relatively long-time periods; and,
- Model habitat use of acoustically active dolphins.

A large number of acoustic products and tools are available that can be used in the above applications. A review of a large range of these products was conducted in 2013 and is given in Sousa-Lima et al. (2013). However, advances in products and tools are occurring rapidly and improved generations of technologies are made regularly. Selecting appropriate products and tools and applying them in an effective experimental design can be undertaken by drawing on knowledge from experienced underwater bio-acousticians that understand the sensitivity and limitations in systems and the requirements of the application.

Future work is required to extend the applications of PAM so that it can be used for:

- Identifying which species of dolphins are being detected; and,
- Monitoring abundance and distribution of vocal and non-vocal animals.

## Products and tools

This project and related work within the region has produced the following products and tools that have potential use by managers and scientists interested in understanding and managing inshore dolphins:

- Mitochondrial DNA sequences and microsatellite genotypes were obtained for snubfin and humpback dolphins in several remote regions for which none were previously available. These data facilitated assessments of population genetic structure in the region and will contribute to future more detailed/broader assessments as further samples are collected.
- Sightings and photo-identification data were obtained for several remote areas in the Kimberley, including measures of relative abundance and catalogues of recognisable individuals. These data

provide a benchmark for future research and monitoring in the region and have been contributed to a state-wide database (WA 'DoFin').

- Several data products were collected which significantly contribute to the application of passive acoustic monitoring to snubfin and humpback dolphins, including:
  - Data on the acoustic repertoire and vocalisation rates of snubfin and humpback dolphins, including a catalogue of whistles for comparison with, and expansion of, similar catalogues obtained from the east coast of Australia.
  - Characterisation of the underwater soundscape for two areas in the western Kimberley: Cygnet Bay and Roebuck Bay, with the latter being a Marine Park.
- The project included several effective collaborations between researchers, indigenous rangers and regional DBCA staff, which have developed positive relationships and the potential for future collaborations.
- Capacity building and skills development has been implemented for Kimberley Marine Park staff and indigenous rangers in best practice techniques for sighting and photo-ID surveys of inshore dolphins.

### Key residual knowledge gaps

Snubfin and humpback dolphins, like many cetaceans, are highly mobile and difficult to observe, and with patchy distributions across large and often inaccessible areas. Consequently, obtaining comprehensive data on their abundance and population genetic structure even at local scales is difficult, expensive, and time-consuming. Our results provide further insight into the genetic structure of snubfin and humpback dolphins in north-western Australian waters, along with information on their relative abundance at selected locations. However, their structure and abundance throughout the Kimberley region as a whole remains unknown, and more detailed information on their demographics, movement patterns, and behavioural ecology is lacking for most locations. Given the challenges and costs associated with collecting data on relative or absolute abundance, it is recommended that future targeted research prioritise the populations and areas of particular conservation and management importance, including: areas with the greatest current or projected exposure to threatening processes; and sites that are suitable for cost-effective long-term monitoring to determine population trend. In keeping with these recommendations, Roebuck Bay should be considered a priority site for monitoring the population status of snubfin dolphins.

Further collection of genetic samples should be similarly selective, although a more strategic approach which seeks to elucidate broad-scale patterns of structure will also be beneficial. In particular, further genetic sampling of animals from Yampi Sound and further into the central and northern/eastern Kimberley, and also within adjacent Northern Territory waters, will facilitate the identification of appropriate management units within the Kimberley region. Traditional Owners and regional wildlife managers are critically important in the collection of data from the remote Kimberley region; wherever possible, targeted research should seek to utilize and further develop their capacity, and make use of opportunities to collect opportunistic data on inshore dolphins alongside their routine operations.

A current limitation to the application of PAM to snubfin and humpback dolphins is a lack of efficient automatic detection algorithms to identify dolphin vocalisations in acoustic datasets, which extends across all areas of study. Existing detection algorithms have been trained on more common species' of dolphins and current algorithms require a significant amount of manual checking to ensure accuracy of detections. Manual checking is highly time consuming and increases the cost of the data analysis, and further efforts to refine detection algorithms is required to improve the efficiency of PAM. In addition, further knowledge on species-specific vocal rates of dolphins in association with behaviours will allow species-specific monitoring of dolphins and the development of dolphin abundance estimation using passive acoustics.

Knowledge gaps remain at all sites investigated in this study, particularly those more remote sites where data collection only provided a limited number of genetic samples and basic information on species composition and relative abundance within a short time period. Unlike the more extensively studied sites of Cygnet Bay and Roebuck Bay, there are currently insufficient data to estimate absolute abundance or residency patterns within

the Prince Regent River or Cambridge Gulf. Similarly, insufficient genetic samples have been collected to examine the connectivity of animals in these areas to those in the western Kimberley.

For those sites with a greater level of understanding, including data which might be further explored to answer additional questions, further information is provided below.

### *Roebuck Bay*

Roebuck Bay provides an excellent location for further study of snubfin dolphins due to the size, accessibility and importance (listed as a Marine Park key asset) of its snubfin dolphin population, in addition to a high level of human use relative to other regions in the Kimberley. While a robust estimate of the number of animals using the northern third of the bay is available, many data gaps of relevance to conservation management remain, including:

1. Habitat use throughout Roebuck Bay and adjacent waters, at various temporal scales, and potential environmental drivers (e.g. depth, seabed habitat, freshwater inputs, tidal phase)
2. Residency patterns, including long-term site-fidelity
3. Ranging patterns of individual dolphins
4. Grouping patterns and social structure
5. Trends in abundance and habitat use
6. Ecological role of snubfin dolphins in Roebuck Bay, including diet
7. Impact of vessel traffic

Items 1-4 (above) could be investigated to some extent with existing data, but would benefit from additional targeted data collection using a range of methods (vessel surveys, PAM, etc.) to result in more robust outputs. For example, stratified sighting surveys with greater spatial and temporal coverage would be required for thorough investigation of items 1 and 2. Items 5-8 would require new data collection specific to those particular research questions; for example, periodic intensive surveys to estimate trends in abundance and habitat use.

### *Cygnets Bay*

Cygnets Bay currently experiences a low level of human use relative to waters such as Roebuck Bay, which is adjacent to a growing town, although vessel traffic may increase in the next few years following completion of sealed road access. With robust population estimates for snubfin, humpback and bottlenose dolphins available for 2012 and 2013, Cygnets Bay represents a good location for ongoing periodic (i.e. every few years) intensive boat-based surveys to examine trends in abundance over time.

With regular sightings of snubfin, humpback and bottlenose dolphins within an area of just 130 km<sup>2</sup>, Cygnets Bay exemplifies the sympatric nature of these three species in northern Australia. Nonetheless, repeated stratified surveys from 2012-2013 suggested the presence of fine-scale habitat partitioning between the species within the study area. An analysis of existing data, ideally substituted with environmental sampling (e.g. seabed imagery, sediment sampling, baited cameras), would provide valuable insight into species-specific habitat preferences, albeit at a small geographic scale. Such a study would be complemented by a comparative study of species diet preferences, involving stable isotope and fatty acid analyses of tissues samples from dolphins, potential prey species and organisms at a variety of trophic levels (would require new sampling).

### *Cone Bay*

While apparently low (i.e. < 30) abundances of snubfin and humpback dolphins within Cone Bay precluded the estimation of robust measures of absolute abundance, there remains a reasonable understanding of the number of dolphins which use the area over a three-week period. Consequently, there is some merit in ongoing periodic boat-based surveys to monitor any substantial changes in relative abundance and longer-term fidelity of individuals to the bay. Such monitoring is made more pertinent by the expansion of aquaculture operations in the area forecast over the coming years. Additional genetic sample collection from this would also improve confidence in existing inferences of population genetic structure in the region.

## 1 Introduction

The Australian snubfin dolphin (*Orcaella heinsohni*, 'snubfin dolphin' hereafter) and Australian humpback dolphin (*Sousa sahulensis*, 'humpback dolphin' hereafter) are two species of small cetacean whose distribution is restricted to shallow coastal and estuarine waters of northern Australia and southern New Guinea (Parra et al. 2002, 2004, Beasley et al. 2005, 2016, Jefferson & Rosenbaum 2014). Available data suggest that both species are discontinuously distributed as small populations of 50-200 (e.g. Parra, Corkeron, et al. 2006, Palmer et al. 2014, Brown et al. 2016), which exhibit site fidelity (Parra, Corkeron, et al. 2006, Cagnazzi et al. 2011, 2013), limited gene flow between populations (Cagnazzi 2011, Brown, Kopps, et al. 2014), and are reliant upon near-shore habitats (Parra, Schick, et al. 2006, Parra & Jedensjö 2014). These characteristics, combined with their slow growth, late maturation, and low reproductive rates, render them vulnerable to human activities which may reduce their ability to survive and reproduce (Marsh et al. 2003).

Basic population data on snubfin and humpback dolphins have increased in recent years (e.g. Brooks & Pollock 2015, Brown et al. 2016). However, a lack of data on the distribution, abundance, trends and threats precludes comprehensive assessment of their conservation status under international<sup>1</sup>, national<sup>2</sup> and Western Australian state criteria, and, consequently, the management of impacts on local populations (Beasley et al. 2012, Woinarski et al. 2014). Due to these data deficiencies, a range of potential (but largely unquantified) threats, and increasing development of coastal areas in recent decades, there have been repeated calls for greater population data to support the conservation and management of inshore dolphins across northern Australia (e.g. Bannister et al. 1996, Ross 2006, Allen et al. 2012, Bejder et al. 2012).

Two key pieces of information that are of particular importance in the conservation and management of wildlife populations are population size and structure. The two are closely related, with an understanding of the number of animals in a population dependent upon determining what constitutes a 'population', i.e. the level of connectivity between individuals across their distribution (Taylor 1997, Wang 2009). For example, a species distributed as a series of small, somewhat isolated population fragments will require different management to a species of the same total abundance, but which is structured as a single, well-connected population (Reed 2004). To this end, analyses of population genetic structure have been widely used to investigate the level of gene flow between adjacent populations, infer migration rates, and assist in the identification of populations which may be classified as discrete 'management units' (Taylor 1997, Palsbøll et al. 2007, Frankham et al. 2010).

The inaccessible nature of much of the coast of northern Australia is in no small part responsible for the paucity of research into snubfin and humpback dolphins that has occurred to date. Further challenges to data collection arise from the often-cryptic behaviour of the species (subtle surfacing behaviour, tendency for boat-avoidance, occurrence in turbid waters). Consequently, there is much merit in the development of scientific techniques that allow autonomous data collection over extended periods. Passive acoustic surveys are increasingly used as either a stand-alone method or for complementing visual survey methods, for detecting cetaceans and identifying abundance and habitat use (Van Parijs et al. 2002, Mellinger et al. 2007, Rayment et al. 2011). This method relies on using the vocalisations of the animals to monitor their movement and behaviour and can be a more cost-effective method than visual surveys. Most applications of passive acoustic monitoring, however, have been to more common species (e.g. harbour porpoise, *Phocoena phocoena*; bottlenose dolphins, *Tursiops* spp.) where there is considerable knowledge of their acoustic repertoire. This method still needs to be developed for lesser studied species such as the snubfin and humpback dolphin, although it does have the potential to provide an important additional tool for managing human impacts on species of high conservation priority.

In the remote waters off the Kimberley coast of Western Australia, estimates of snubfin and humpback dolphin population size and structure are currently limited to more accessible areas in the western Kimberley (Brown, Kopps, et al. 2014, Brown et al. 2016). A single study of genetic connectivity revealed limited gene flow

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<sup>1</sup> International Union for Conservation of Nature (IUCN) Categories and Criteria for Species Status Assessment (IUCN 2012).

<sup>2</sup> Threatened Species Scientific Committee Guidelines for assessing the conservation status of native species according to the *Environment Protection and Biodiversity Conservation Act 1999* (TSSC 2015).

between snubfin dolphins sampled at Roebuck Bay and Cygnet Bay (Brown, Kopps, et al. 2014), yet nothing is known of gene flow between these local populations and animals occurring further east in the central and northern Kimberley. With the recent establishment of several Marine Parks along the Kimberley coast, including snubfin and humpback dolphin assets within their management plans, there is a pressing need for an improved understanding of these species' occurrence in the region and their level of interactions with human activities such as vessel traffic, commercial fishing and coastal habitat modification.

Here, we present the findings of research which builds upon that of several existing projects aimed at improving the understanding of snubfin and humpback dolphins in north-western Australian waters, and developing new methods to enhance their study. Specifically, we use a combination of techniques to address objectives under the following research areas:

#### *Population genetic structure*

Collect tissue samples from free-ranging dolphins and use nuclear and mitochondrial genetic markers to examine population genetic structure and gene flow between several locations within the Kimberley region.

#### *Relative abundance*

Conduct boat-based surveys and photo-identification to obtain information on species composition, group size and composition, and encounter rates of snubfin and humpback dolphins at several locations in the Kimberley region.

#### *Passive acoustic monitoring*

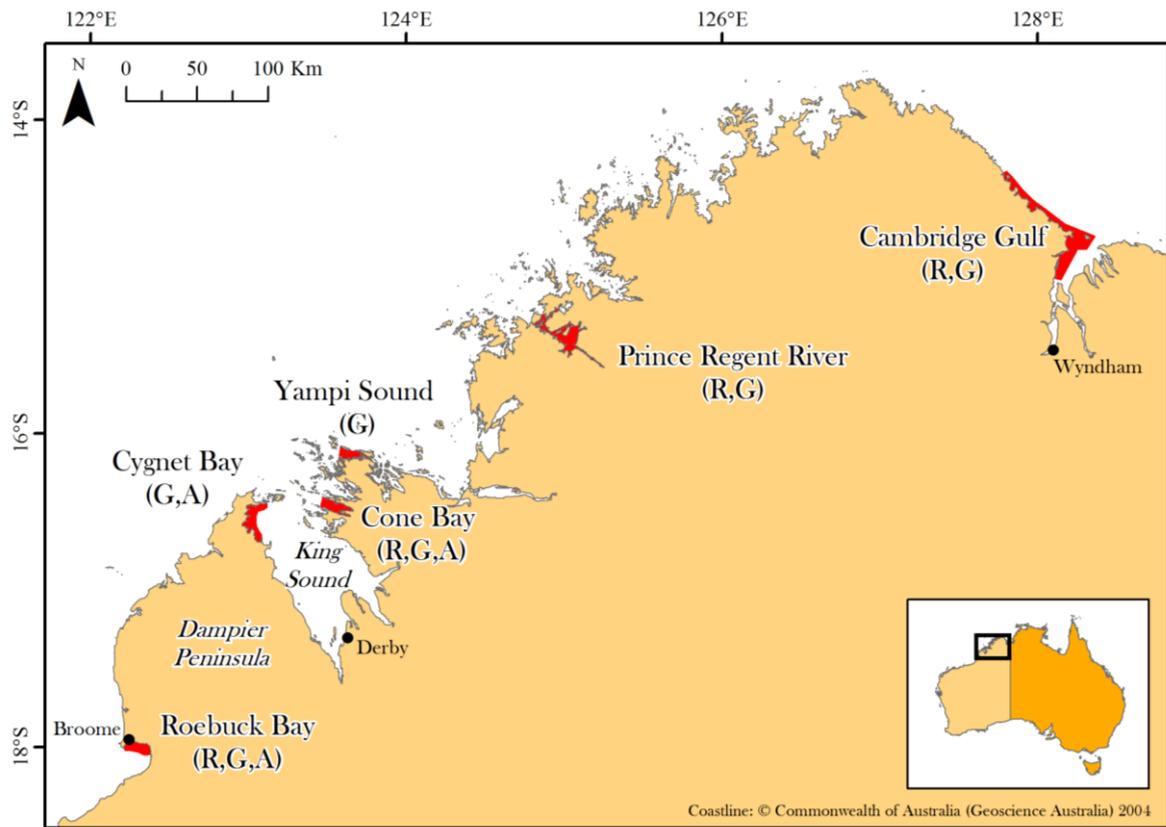
Develop Passive Acoustic Monitoring (PAM) techniques for snubfin and humpback dolphins within the Kimberley by: (i) developing an understanding of the underwater soundscape; and (ii) correlating acoustic and visual observations of dolphins to validate species presence, identify acoustic repertoire and examine vocalization rate and group size/behaviour relationships.

Our findings extend the geographic scope of quantitative data on snubfin and humpback dolphins, provide new insights on their population genetic structure in the region, and make valuable progress towards the development of PAM for application to these species. These results inform the conservation and management of the species in the region, including the development of monitoring within Marine Parks.

We note here that Indo-Pacific bottlenose dolphins (*Tursiops aduncus*, 'bottlenose dolphins' hereafter) also occur across the Kimberley and Pilbara coastlines, often in sympatry with snubfin and humpback dolphins (Allen et al. 2012, 2016, Brown et al. 2016). While we collected and report on photo-identification data and group characteristics from encountering bottlenose dolphin groups, details on their relative abundance and population size estimates from some locations can be found in Brown et al. (2016). The focus of this report remains on the lesser-known, less-widely distributed snubfin and humpback dolphins.

## **2 Materials and Methods**

The Kimberley coast of north-western Australia is long and intricate, with complex habitats subject to large, semi-diurnal tides of up to 10 m range (Cresswell et al. 2011) (Figure. 2.1). Much of the area is only accessible by boat, helicopter or seaplane, with sealed roads to the coast limited to Broome and Derby in the west, and Wyndham in the east. Consequently, the selection of study sites was strongly influenced by accessibility, in addition to the presence of an established logistical base, prior knowledge or reports of the occurrence of snubfin and humpback dolphins, and the specific project objectives being addressed (see below).



**Figure 2.1.** The Kimberley region, illustrating project study sites where data on dolphin relative abundance (R), population genetic structure (G) and/or passive acoustic monitoring (A) are presented here.

## 2.1 Population genetic structure

Under this project, the collection of genetic samples was attempted at: (1) Cone Bay (Buccaneer Archipelago) in September 2014 and 2015; and, (2) in the Cambridge Gulf (north Kimberley coast) in August 2016. Participation by AMB in a weeklong Marine Park patrol aboard the *PV Worndoom* facilitated limited additional sampling from the Prince Regent River (central Kimberley coast) in September 2016<sup>3</sup>. Limited additional genetic samples were also obtained through opportunistic collection at Yampi Sound (Buccaneer Archipelago) during a non-WAMSI collaborative survey by AMB and the Dambimangari Rangers in October 2016. We further supplemented the sample size by: (1) incorporating snubfin dolphin samples collected at Roebuck, Cygnet and Cone Bays by SJA, AMB and DT under previous projects (see Brown, Kopps, et al. 2014), including a significant proportion collected in April 2014 which had not yet been analysed; and, (3) incorporating published genotypes from humpback dolphins sampled at the North West Cape and Dampier Archipelago (Brown, Kopps, et al. 2014). The latter facilitated a preliminary investigation of the genetic connectivity of humpback dolphins between the current focal region of the Kimberley and sites further west in the Pilbara region.

Genetic (skin tissue) samples were obtained from free-ranging dolphins using the well-established PAXARMS biopsy darting system from small research vessels (Krützen et al. 2002). During the collection of sighting and photo-identification data, trained operators assessed animals for their suitability for biopsy darting. Successful sample collection requires close proximity to an individual (i.e. five to 20 metres), a predictable surfacing pattern, and calm sea conditions. No attempt was made to sample calves or adults with neonate calves. As such, darting was conducted opportunistically in suitable conditions only.

<sup>3</sup> The original project agreement specified two sites: Cone Bay and One Tree Beach (Admiralty Gulf). For logistical reasons, One Tree Beach was subsequently changed to the Cambridge Gulf, and the additional site of the Prince Regent River was added through a collaboration with the Lalang-garram/Camden Sound Marine Park management team.

Tissue samples were stored in either 100% ethanol or saturated NaCl/20% dimethyl sulfoxide (Amos & Hoelzel 1991) and, where possible, kept frozen until the time of analysis. Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's instructions. Sex was determined molecularly using sex chromosome-specific primers. Loci ZFX and SRY (Gilson et al. 1998) were coamplified in a single PCR reaction. PCR products were run on a 1.5 % agarose gel and sex determined based on the number of different fragments amplified.

We examined population genetic structure using nuclear (microsatellite) and mitochondrial (mtDNA) markers. While both can be used to investigate the genetic connectivity of sampled populations, they provide different information. Microsatellites are bi-parentally-inherited, thus inferring the level of gene flow from both males and females among subpopulations. MtDNA markers, by contrast, are maternally-inherited; they infer only on female-mediated gene flow and can reveal patterns of natal site fidelity. Comparing evidence of gene flow between mtDNA and microsatellites from the same sampled populations can, therefore, provide insight into sex-specific dispersal patterns.

Mitochondrial DNA (mtDNA) haplotypes were assigned based on a 382 base-pair (bp) and 391 bp sequence for snubfin and humpback dolphin, respectively. The fragment was amplified by the primers dlp1.5 and dlp5 (Baker et al. 1993). The PCR conditions described in Bacher et al. (2010) were followed. MtDNA sequences were aligned with the software Geneious R10.0.1 (Biomatters) and haplotypes were assigned with the software DNAsp 5.10 (Librado & Rozas 2009).

Fourteen microsatellite loci were amplified in four 10 µl volume multiplex PCRs using Qiagen Multiplex Kit™ (Qiagen). The microsatellite markers used were: DirFCB4, DirFCB5 (Buchanan et al. 1996), LobsDi\_7.1, LobsDi\_9, LobsDi\_19, LobsDi\_21, LobsDi\_24, LobsDi\_39 (Cassens et al. 2005), SCA9, SCA22, SCA27, SCA39 (Chen & Yang 2009), TexVet5 and TexVet7 (Rooney et al. 1999). The PCR conditions as described in Frère et al. (2010) were followed. The single stranded PCR products were run on an ABI 3730 DNA Sequencer (Applied Biosystems). Alleles were scored with Geneious R10.0.1 (Biomatters). Duplicate samples, i.e. samples that were genotyped for at least 10 microsatellite loci and matched 95%, were identified using the Microsatellite Toolkit (Park 2001) and, from these, the sample with the most complete genotype was retained. Microsatellites were checked for Hardy-Weinberg equilibrium and linkage disequilibrium in GenePop (Rousset 2008).

Several measures of population differentiation were calculated for the sampled study sites. Pairwise genetic distance  $F_{ST}$  values (for microsatellites and mtDNA) were calculated in Arlequin (Excoffier et al. 2005). The genetic structure between populations was examined in STRUCTURE [version 2.2.3 (Pritchard et al. 2000, Falush et al. 2003)] using an admixture model with loc-prior information on sampling location. The model was run with a burn-in length of  $10^5$  following by  $10^6$  Markov chain Monte Carlo (MCMC) steps and with 10 independent interactions for each potential number of genetic clusters ( $K = 1$  to 7). The most likely number of genetically homogeneous clusters (if  $K$  greater than two) was determined by the greatest mean posterior probability distribution (mean of  $\ln P(D)$  from the 10 iterations for each  $K = 1-7$ ) and calculating  $\Delta K$ , an ad-hoc statistic proposed by Evanno et al. (2005).

Contemporary migration rates between sampled populations were calculated in BayesAss 1.3 (Wilson & Rannala 2003) using  $10^7$  iterations, a burn-in length of  $10^6$  and a sampling interval of 1,000 steps. Three runs per species with different seeds were performed to confirm that similar mean posterior migration rates and 95% confidence intervals were obtained.

## 2.2 Relative abundance

Data on the relative abundance of dolphins was collected concurrent to genetic sampling at Cone Bay in September 2014, the Cambridge Gulf in August 2016, and the Prince Regent River in September 2016 (Figure 2.1). Additional opportunistic photo-identification data was collected during acoustic data collection activities at Cone Bay in September 2015.

Study sites were surveyed using a research vessel of 5-6 m in length with three to five observers. The Cone Bay and Prince Regent River study sites were surveyed by following pre-determined transect routes of c. 45 km length, approximately configured in a zig-zag pattern to provide even coverage of the area. Weather permitting, the transect routes were completed a minimum of three times. To reduce bias in our ability to detect dolphins, we aimed to conduct the vast majority of survey effort in Beaufort sea states  $\leq$  two and wave height  $\leq$  0.3 m. Transects were completed in the shortest possible time at a survey speed of 5-6 knots. If a transect could not be completed in a single day (due to deteriorating sea conditions or low light), effort was

paused and resumed from that location at the next available opportunity (typically the following day). At the Cambridge Gulf study site, locating animals suitable for biopsy darting was prioritized over even coverage of the study area, and, therefore, survey effort did not follow a pre-determined route.

Observers searched for dolphins from the front half of the vessel. Upon sighting dolphins, we departed from the survey route and approached the dolphin group to record date, time, GPS location, species, and group size, composition and behaviour. Two to three observers with digital SLR cameras attempted to obtain multiple photographs of the dorsal fins of all dolphins present so that at least one good-quality image of each individual present was obtained. We defined a 'group' as one or more dolphins within 100 m of any other group member and involved in the same or similar behavioural activity (Bräger 1999, Parra, Corkeron, et al. 2006). Group size was estimated in the field, and subsequently validated with photo-identification data where available. There was occasionally uncertainty over the exact group size, and in such cases a minimum, maximum and best estimate were recorded, with the best estimate being used to generate summary statistics.

Daily vessel GPS tracks were assigned on/off effort values in accordance with effort logs recorded in the field<sup>4</sup>, and interpolated to lines of effort within a Geographic Information System (GIS, software used: ArcGIS 10.4, ESRI). The length (km) of survey effort was calculated in the GIS and summarised by day and transect. The total number of dolphins (including dependent calves) observed on a given transect/day was then divided by the km of survey effort. Individual dolphins sighted more than once within a single transect/day (indicated by photo-identification) were not counted a second time. Per-transect/day dolphins/km values were summarised across all transects/days within a data collection period to provide a standardised measure of relative abundance as the mean ( $\pm$  SE) dolphins per km survey effort.

### 2.3 Photo-identification

Individual dolphins were identified from photographs based on nicks and notches on the leading and trailing edges of the dorsal fin, resulting in a catalogue of individuals for each study site (Würsig & Jefferson 1990). Only dolphins with distinctive dorsal fin features captured in an image of sufficient quality were assigned individual IDs (Urian et al. 1999, 2015). The assessment of dorsal fin distinctiveness and image quality was performed by a single observer (AMB) based on published protocols (Urian et al. 1999, 2015, Rosel et al. 2011) and the underlying assumption that the least distinctive individual should be readily identifiable from the lowest quality image used in the analyses (Nicholson et al. 2012, Urian et al. 2015). For each site, we also calculated the proportion of dolphins encountered for which acceptable quality photo-ID images were obtained, to provide an indication of the relative success of photo-ID as a technique at each site.

### 2.4 Passive Acoustic Monitoring (PAM)

PAM of snubfin and humpback dolphins was conducted at three sites within the Kimberley; Cygnet Bay during May 2014, Roebuck Bay during July and September/October 2014 and Cone Bay during September 2015 (Figure 2.1). This fieldwork was a smaller component of a larger ongoing study led by JS which was funded by the Australian Marine Mammal Centre to develop PAM methodology for these species Australia-wide. PAM of snubfin and humpback dolphins requires a coordinated approach involving simultaneous visual and acoustic observations of the dolphins to document the species vocal repertoire and ground-truth the number and types of vocalisations with variables such as group size and behaviour. A focus on intra- and interspecific variation of the different vocalisations among different populations of coastal dolphins in Australia will enhance our capacity to use PAM at various spatial scales to detect these species. The fieldwork setup and methodology was slightly varied in each of the three different fieldwork locations dependent on the species present and whether the aims were soundscape monitoring, relating detections to visual observations of dolphins, or dolphin vocal repertoire in relation to behaviour. Given the considerable knowledge gaps in humpback and snubfin dolphin distribution and abundance throughout the Kimberley, the PAM fieldwork relied on *a priori* knowledge from previous surveys undertaken in the region to identify areas of high dolphin density and maximize dolphin encounters.

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<sup>4</sup> While GPS tracks were recorded during 2015, this was not considered survey effort as generally only two observers were present and the focus of the work was to collect acoustic recordings.

## 2.4.1 Soundscape monitoring

### Roebuck Bay

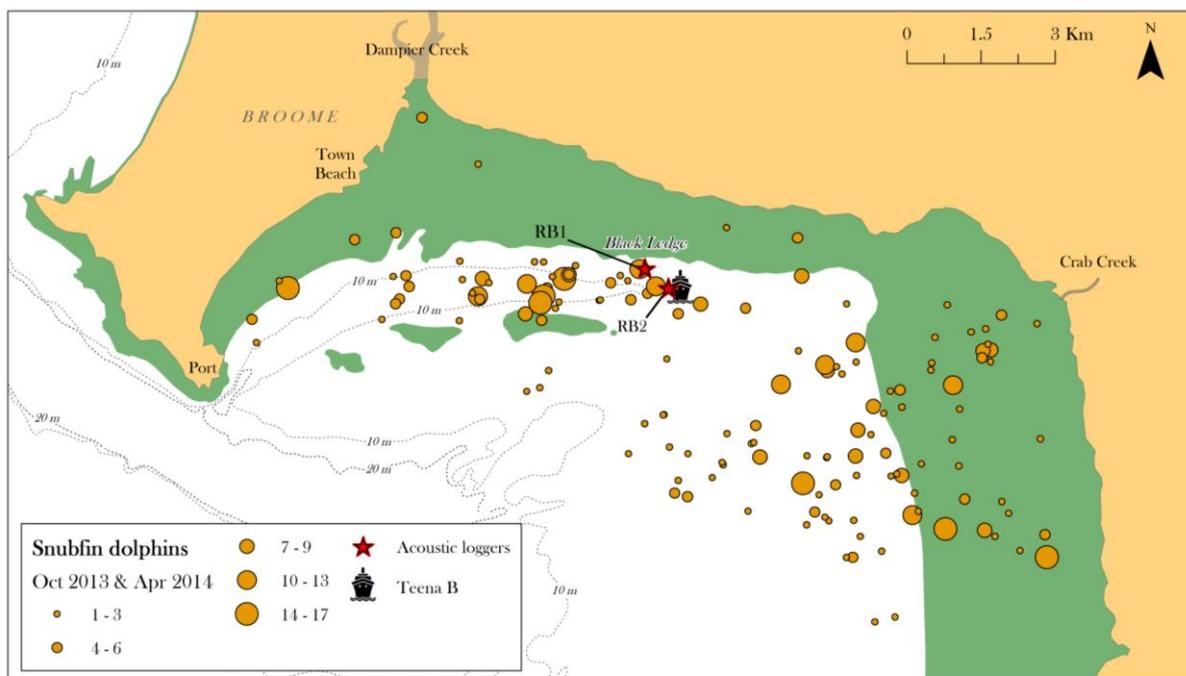
Roebuck Bay is a large tropical, marine embayment, approximately 550 km<sup>2</sup> in size. It is bordered by mangroves and sandy beaches, and has a number of tidal creeks that are a source of freshwater input during the rainy season. Roebuck Bay is located in a hot semi-arid climate, which experiences a wet and a dry season. The wet season occurs during the austral summer, while the dry season occurs typically between May and November. The bay experiences very large semi diurnal tides of up to c. 10 m in range and averaging 5.7 m range.

During July and September/October 2014, high-frequency acoustic receivers were deployed in Roebuck Bay between Black Ledge and a cyclone mooring area to record the soundscape (Figure 2.2). This location was known to be an area of high use by snubfin dolphins for foraging and socialising (Thiele 2010).

The acoustic receiver consisted of a high-frequency sea noise logger built at CMST (Centre for Marine Science and Technology; Curtin University). The receiver had a custom-built impedance matching pre-amplifier, a programmable channel amplifier, and a Wildlife Acoustics Inc. Songmeter™ motherboard. The sampling frequency for recordings was 192 kHz (Table 2.1). A high-pass filter filtered out low-frequency noise below 8 Hz so that the dynamic range of the recorder was enhanced. The logger recorded 10 min every 15 min. Recordings were written to four 128 GB SD cards in the acoustic recorder. The electronics associated with the recorder were placed in a waterproof steel housing with the hydrophone mounted externally.

The same receiver was deployed twice. The first time to record the soundscape within Roebuck Bay between July 4 to July 31 (recording RB1 in Table 2.1) and the second time between September 24 to October 6 (recording RB2).

The frequency response of the acoustic receiver was calibrated before deployment and after recovery by inputting white noise of a known level in series with the hydrophone, and then correcting the recorded signal for the hydrophone sensitivity. The hydrophone signal was amplified using an impedance matching pre-amplifier of 20 dB gain and a channel amplifier with gain setting of 24 dB. Signals were high-pass filtered with a roll-off starting at 3 Hz. This reduced the naturally high levels of low-frequency acoustic and static pressure fluctuations, and thus increased the dynamic range of the acoustic recording system. The amplified signals were low-pass filtered by an anti-aliasing filter and then fed to a 16-bit analogue-to-digital converter (ADC).

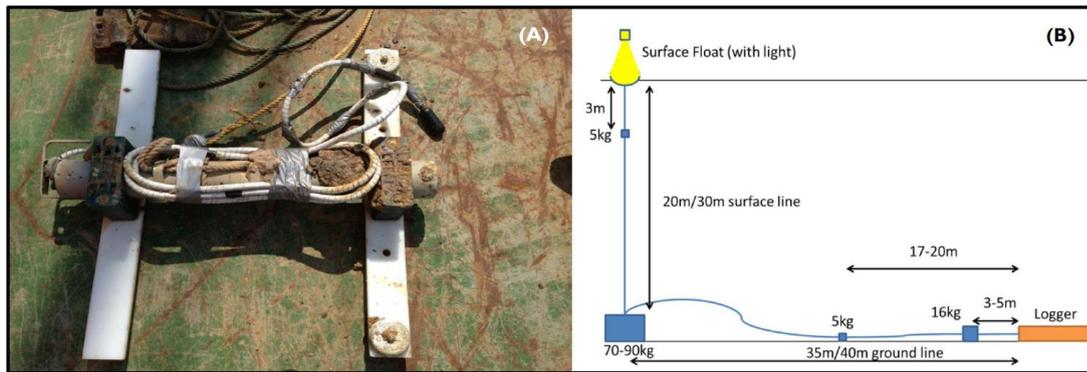


**Figure 2.2.** Roebuck Bay, WA, study area, illustrating: acoustic logger deployment locations (RB1 and RB2 – see Table 2.1); location of moored vessel Teena B from which visual observations were made in 2014 (See Section 2.4.3). Locations and sizes of snubfin dolphin groups recorded during previous visual surveys in October 2013 and April 2014 (Brown, Bejder, et al. 2014) are also shown to demonstrate known dolphin distribution relative to the acoustic data collection locations.

**Table 2.1.** Acoustic logger recordings obtained using loggers deployed on the sea bed in Roebuck Bay (WA), their positions, sample rates, and recording schedules.

Deployment / Dataset	Location		Recording Period			Sampling Schedule		
	Latitude	Longitude	Start	End	Days	Sampling Rate (kHz)	Total Gain (dB)	Duty Cycle (min)
RB1	17° 59.215	122° 17.566	4/07/2014	31/07/2014	27	192	44	10 every 15
RB2	17° 59.430	122° 17.837	24/09/2014	6/10/2014	12	192	44	10 every 15

The acoustic receiver was deployed on a mooring designed to decouple the main riser leading to the surface float from the logger to reduce noise from the chains on the main mooring (Figure 2.3). The unit was deployed on the seabed and attached to a surface mooring as depicted in Figure 2.3. Deployment depths were approximately 7m (Lowest Astronomical Tide).



**Figure 2.3.** Photograph of logger after retrieval (A), and schematic showing the acoustic logger set-up utilised in Roebuck Bay (WEIGHTS AND LINE LENGTHS VARY ACCORDING TO DEPLOYMENT; B).

Prominent sound sources were identified by reviewing spectrograms created in CHORUS (Gavrilov & Parsons, 2014), a toolbox developed at CMST (Curtin University) in Matlab (Version R2014a, The MathWorks Inc.). Spectrograms were created by first applying a Fourier transformed to recordings in 1 s windows. A time series of power spectral densities (PSDs) was produced. Broadband noise levels were calculated for each 10-min recording.

### Cygnets Bay, 2014

Cygnets Bay is a shallow, tropical marine embayment of c. 200 km<sup>2</sup> within King Sound in the western Kimberley region. The area is subject to large semi-diurnal tides of up to 10 m in range, and supports a variety of coastal habitats within its constituent smaller bays and headlands, including: rocky, sandy and mangrove intertidal shores, tidal creeks, and subtidal reefs, sandbanks and muddy habitats. The same acoustic receiver was deployed at Cygnets Bay as the one used in Roebuck Bay (see Figure 2.3 and Table 2.2 for acoustic logger location and schedule). In Cygnets Bay, the receiver was deployed to record the soundscape between May 6 and May 26 (recording SB1; Table 2.2). Deployment and analytical methods were the same as for Roebuck Bay.

**Table 2.2.** Acoustic logger recordings obtained using loggers set on the sea bed in Cygnets Bay (WA), their positions, sample rates, and recording schedules.

Deployment/ Dataset	Location		Recording Period			Sampling Schedule		
	Latitude	Longitude	Start	End	Days	Sampling Rate (kHz)	Total Gain (dB)	Duty Cycle (min)
SNR55	16° 34.736	123° 00.145	6/05/2014	26/05/2014	20	192	44	10 every 15

## 2.4.2 Vocal detections in relation to dolphin occurrence

### *Roebuck Bay*

Visual observations were timed to overlap acoustic logger recording RB1 (the site selected to have high dolphin occurrence), and were conducted on all good weather days between 6 and 29 July 2014. A c. 5 m outboard vessel traveling at 5 knots was used to make observations along transects designed to sample the full range of nearshore environments in Roebuck Bay. A basic systematic random sampling design (Figure 2.4) using Distance (6.0) was used to design a grid of line transects within two strata<sup>5</sup>. The first stratum (Stratum 1, Table 2.3) covered a large area with transects spaced 2 km apart, while the second stratum covered a much smaller area with transects spaced 1 km apart (Stratum 2, Table 2.3). The small stratum was designed to overlap with the locations of the acoustic logger.

**Table 2.3.** Strata statistics of systematic random sampling design used in Roebuck Bay in July 2014.

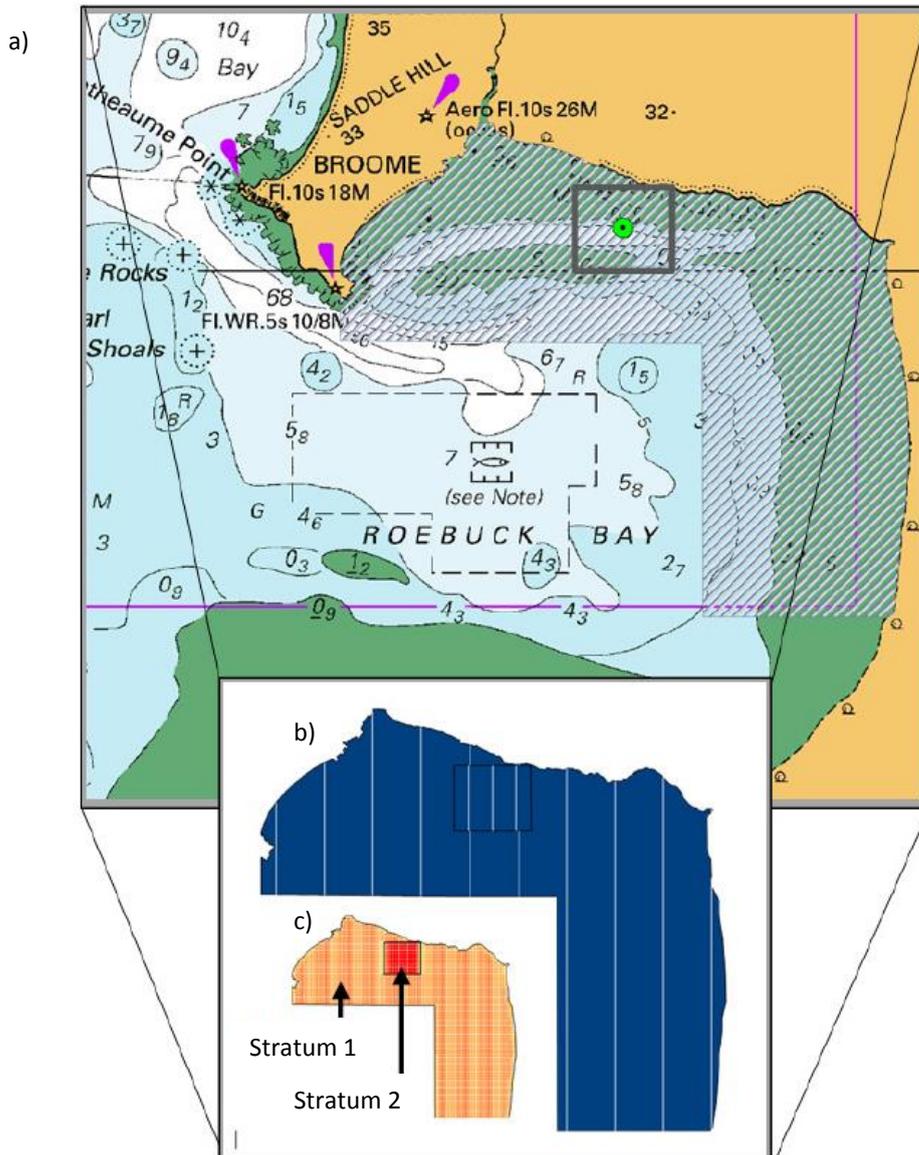
Strata	Description	Area	Mean coverage probability (assuming sampler width = 0.5 km)	Mean on-effort total transect length (km)	Number of transects
1	Broad coverage	159.6 km <sup>2</sup>	0.49	80.1	10
2	Acoustic logger coverage area	8.6 km <sup>2</sup>	0.95	8.6	3

The order in which transects were conducted was randomly selected on the day, but was also dependent upon sea conditions in different parts of the bay. On any survey day, if there was a small weather window to conduct surveys, priority was given to Stratum 2. Where possible, surveys in both strata were undertaken on the survey day. If time and weather permitted Stratum 2 was surveyed a second time within a day.

Two observers searched ahead (90 degrees, one to port and the other to starboard) of the vessel. Observers also checked behind the vessel regularly. Upon making a sighting of dolphins, the vessel stopped to allow data collection on distance and angle to the group, group composition, group size, group predominant behaviour, GPS position, water depth, sea surface temperature, Beaufort sea state, glare score, and number of vessels within visual range. Vessels sighted were photographed with the horizon captured within the image for distance estimation (not presented here). Dependent upon the behaviour of the animals (readily approachable or travelling fast) the dolphin group was approached for photo-identification (which are not presented here). The aim was to photograph all individuals in a group, but this was balanced by the need to complete transect lines and grids in a timely manner to avoid temporal effects on the distribution of animals in the grid area. After collection of photo-ID, the transect was resumed where it had been left.

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<sup>5</sup> The current design differed from that used by AB in October 2013 and April 2014 which focused on mark-recapture abundance estimation.



**Figure 2.4.** Systematic random sampling design used in Roebuck Bay in July 2014, showing Stratum 1 covering a range of nearshore environments, Stratum 2 covering the location of the acoustic logger, and the acoustic logger position (green circle, shown in a). Transects (shown in b) were designed to have higher coverage probability (shown in c).

During boat-based surveys, hand-held acoustic recordings were made at the beginning and end of transects, and when a group of dolphins had been approached for photo-ID and the vessel motor could be switched off without losing sight of the animals. Recordings were made with the survey vessel engine switched off.

Acoustic recordings were made using a hand-held RESON TC4034 hydrophone deployed over the side of the survey vessel at approximately 1.5 m depth (Figure 2.5). The hydrophone sensitivity was  $-217.3$  dB re  $V/\mu\text{Pa}$ . The underwater noise being measured was amplified using a RESON VP-1000 pre-amplifier with gain settings to suit the environment, and the data recorded on a SoundDevices 722T digital recorder. The digital recorder was set at a sampling frequency of 192 kHz with internal gain of 18 dB. The recording system (Figure 2.5) was calibrated prior to measurements by recording a white noise signal of known level and input in series with the hydrophone. The calibration provided the frequency response of the system.



**Figure 2.5.** Hand-held recording system used for in-situ, vessel-based recordings.

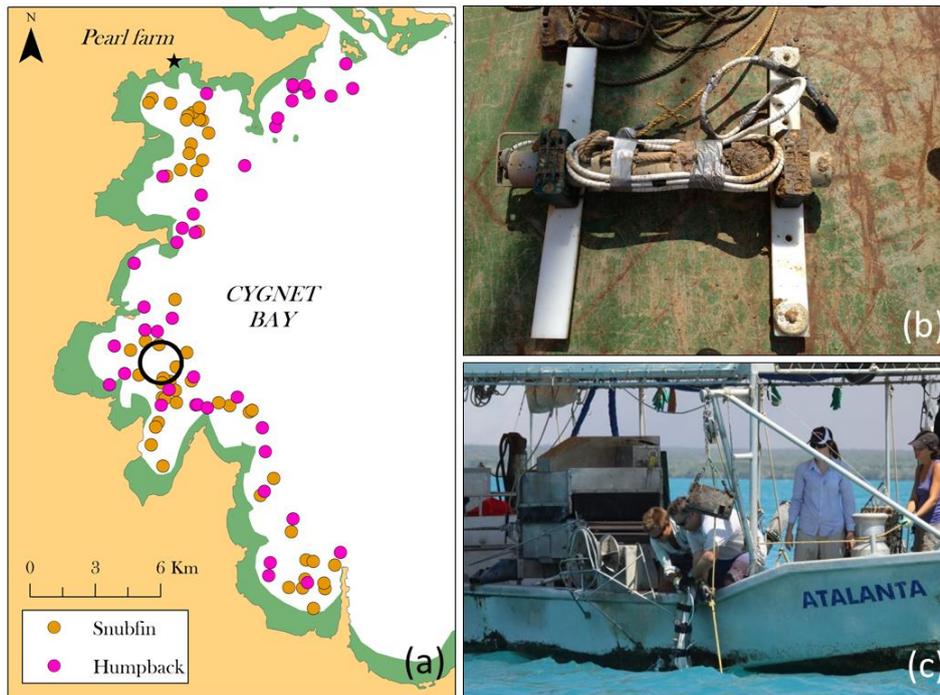
As for the acoustic receiver mounted on the seabed, the hand-held recording system was calibrated prior to measurements by recording a white noise signal of known level and input in series with the hydrophone. The calibration provided the frequency response of the system. Hand-held acoustic recordings were manually reviewed in Adobe Audition (Vr 8.1.0.162) to identify the presence, number and time of dolphin sounds within each file.

### 2.4.3 Dolphin acoustic repertoire, behavioural context and geographic variation

#### *Cygnets Bay*

Based on visual surveys and abundance estimates undertaken by AMB in 2012/13, an area of high-use by snubfin and humpback dolphins within Cygnets Bay (Lat -16.579, Lon 123.002) was identified (Figure 2.6). A CMST sea noise logger equipped with a high and low frequency hydrophone was deployed at this location (Figure 2.6b, c) in May 2014 to monitor the underwater soundscape to enable characterization of the ambient noise and record dolphin acoustic signals. The acoustic datalogger was scheduled to record for 10 minutes at 15 minute intervals for the duration of the fieldwork.

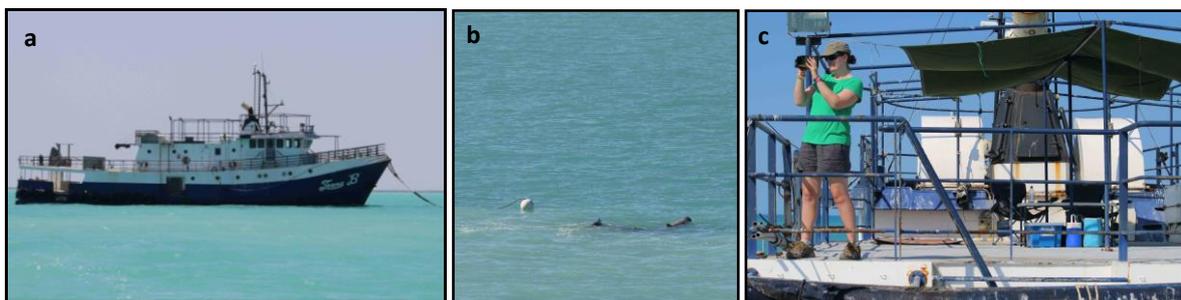
Vessel based surveys were undertaken in Cygnets Bay using a 5.6 m research vessel, with two observers on board conducting acoustic recordings and visual observations of snubfin and humpback dolphins independent of the acoustic datalogger. Although both species were present in the area, there was a lower relative abundance of humpback dolphins compared to snubfin dolphins and consequently data collection predominantly focused on snubfin dolphins. The main aim of the fieldwork was to conduct visual observations of both snubfin and humpback dolphins to validate the vocal repertoires of these species. Acoustic recordings from the boat were obtained using a HTI-96 MIN (2 Hz to 30 kHz) hydrophone and Zoom H4N hand-held acoustic recorder (48 kHz bandwidth, 32-bit stereo) when dolphins were within approximately 100m of the vessel. The second observer obtained photo-identification of the dolphins using a Canon DSLR camera and recorded the behavior and group size of the focal group.



**Figure 2.6.** (a) Location of the deployed acoustic datalogger (black circle) in Cygnet Bay 2014 and inset map of the sightings of snubfin and humpback dolphins (from Brown et al. 2016), demonstrating the high density of dolphins in the vicinity of the acoustic datalogger; (b) a photograph of the acoustic datalogger; and, (c) the retrieval of the acoustic datalogger from the seabed.

### Roebuck Bay

Fieldwork was undertaken in Roebuck Bay onboard Clipper Pearl's vessel *Teena B*, a moored 35 m pearling mother ship with an elevation of 7.5 m above the water line. The *Teena B* (Lat -17.997, Lon 122.298; see Figure 2.2 and 2.7) was located within an area of the inner coastal channel that supports a high density of snubfin dolphins (based on previous survey data provided by AMB and DT) and was used as an elevated survey platform to conduct visual observations of snubfin dolphins within the immediate vicinity of the vessel in September-October 2014. The aim of the fieldwork was to conduct visual observations of dolphins and obtain their locations which was used to validate the vocal repertoire of snubfin dolphins, determine detection limits of their vocalisations and correlate vocalisation rates with group size/behaviour.



**Figure 2.7.** Photograph of (a) the *Teena B* which was used as the dolphin visual observation platform, (b) snubfin dolphins swimming close to one of the three deployed acoustic recorders around the vessel to collect dolphin acoustic data and (c) observations on the top deck of the *Teena B*.

The fieldwork consisted of the deployment of a moored CMST sea noise logger on the seabed equipped with a low frequency hydrophone to monitor the underwater soundscape to enable characterization of the ambient noise (see Section 2.4.1 for a detailed description). Three self-contained SoundTrap 300 HF (20 Hz to 150 kHz bandwidth) acoustic recorders were deployed on temporary moorings within approximately 250 m of the

Teena B to continuously record dolphin acoustic signals. Visual observations of dolphins in close proximity to the SoundTrap recorders were conducted on the top deck of the Teena B by two observers using a Canon DSLR camera with a mounted Canon GPS receiver (Canon GP-E2), Bushnell 10x50 binoculars and a Sony digital camcorder to record group size, group composition and behaviour of dolphins when they are around the SoundTrap recorders. The DSLR camera obtained time stamped photographs of the dolphins with associated calibrated bearing data. Using the program VADAR (developed by Dr Eric Kniest of the University of Newcastle) and elevation of the observer, the GPS position and bearing from the observer recorded by the camera can be used to determine the location of the dolphins when at the surface.

### *Cone Bay*

Fieldwork was undertaken in Cone Bay in September–October 2015, on and around Turtle Island (Lat -16.490, Lon 123.527), with the support and logistical assistance of Marine Produce Australia (see Figure 2.1 and A2.1 [Appendix 2]). The main objective of the fieldwork in Cone Bay was to collect broadband acoustic data on humpback dolphins which are in greater abundance than snubfin dolphins at this site. The aims were to identify the acoustic repertoire of this species and correlate acoustic vocalisations with group size and behaviour. To achieve these aims, three SoundTrap 300 HF (20 Hz to 150 kHz bandwidth) acoustic recorders were deployed around the sea cages of the barramundi fish farm, operated by Marine Produce Australia. Land-based observations of dolphins in close proximity to the SoundTrap recorders were conducted by two observers using a Canon DSLR camera with a mounted Canon GPS receiver (Canon GP-E2) and Bushnell 10x50 binoculars to record group size, group composition and behaviour of dolphins when they were around the SoundTrap recorders (Figure 2.8). The SLR camera obtained time stamped photographs of the dolphins with associated calibrated bearing data. Using the VADAR program, the GPS position and bearing from the observer recorded by the camera can be used to determine the location of the dolphins when at the surface.



**Figure 2.8.** Photograph of the land-based observation platform on Turtle Island overlooking the fish farm sea cages (left), and a dolphin leaping near the sea cages and acoustic recorders (right; photo: Josh Smith).

A 6.0 m centre console boat was used to maximise encounters with dolphins away from the sea farm cages and obtain mobile acoustic recordings of dolphins encountered opportunistically. Upon encountering a group of dolphins, the group size, behavior and photo-identification of individuals were recorded and an assessment was made regarding the suitability (i.e. behaviour and movement patterns) of obtaining acoustic recordings. A SoundTrap acoustic recorder was used for mobile acoustic recordings and was attached to a 25cm diameter polystyrene float by marine grade rope. When acoustic recordings with good signal to noise ratio of dolphin vocalizations were considered feasible, the research vessel was maneuvered into a position that would maximize the encounter time of the acoustic recorder with the group of dolphins without repositioning it. This often meant deploying the acoustic recorder upwind of the dolphins in windy sea conditions or ahead of the group of dolphins when they were exhibiting consistent behaviour.

### 3 Results

#### 3.1 Population genetic structure

After removing genetic samples where either DNA failed to amplify or microsatellite data was largely incomplete (i.e. missing data for >50 % of loci), a total of 106 snubfin and 54 humpback dolphin samples were included in the microsatellite analyses (Table 3.1). For snubfin dolphins at Roebuck and Cygnet Bay sites, sample sizes for mtDNA were slightly lower than that for microsatellites; this was due to small, older samples (collected from 2008-2013 and not analysed for mtDNA at the time) no longer containing sufficient DNA concentrations for analysis. Sample sizes were also lower for humpback dolphins at the North West Cape and Dampier Archipelago, where available sequences from previous analyses were utilised (Brown, Kopps, et al. 2014).

On average, we genotyped 95% of loci per individual. A total of 11 polymorphic loci were included in the analyses for snubfin dolphins, while three loci were excluded: Lobs7.1 exhibited missing data for over 50% of samples; LobsDi\_9 was monomorphic; and, LobsDi\_39 exhibited a homozygosity excess for all sites, suggesting the presence of null alleles and/or inbreeding. For snubfin dolphins, all 11 retained loci were in Hardy-Weinberg Equilibrium (HWE) after Bonferroni correction, with the exception of two loci (LobsDi\_24 and SCA39) sampled at Roebuck Bay where there was evidence of homozygosity excess. As this deviation from HWE was found in only one sampled population, the loci were included in the analyses.

A total of 13 polymorphic loci were included in the analyses for humpback dolphins; TexVet5 was monomorphic. All 13 retained loci were in HWE after Bonferroni correction, with the exception of loci SCA22 and SCA27 in samples at Cygnet Bay and locus LobsDi\_39 in samples at Cone Bay, where there was evidence of homozygosity excess. As these deviations from HWE affected only one sampled population each, the loci were included in the analyses.

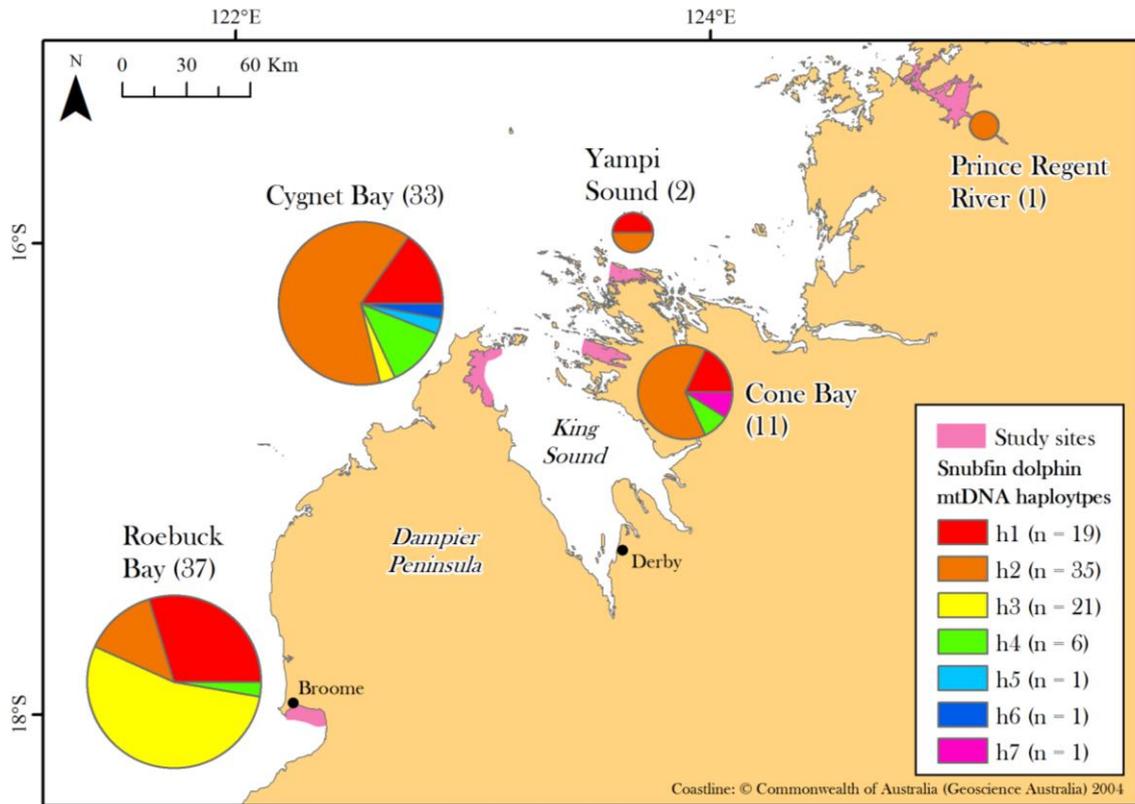
**Table 3.1.** Genetic sample sizes included in the analyses by species and genetic markers.

Site	Snubfin dolphins		Humpback dolphins	
	mtDNA	microsatellites	mtDNA	microsatellites
North West Cape*	-	-	13	18
Dampier Archipelago*	-	-	13	17
Roebuck Bay‡	36	53	-	-
Cygnet Bay**	34	40	6	6
Cone Bay	11	10	12	12
Yampi Sound	2	2	-	-
Prince Regent River	1	1	-	-
Berkeley River / Cambridge Gulf†	-	-	1	1
<b>Total</b>	<b>84</b>	<b>106</b>	<b>45</b>	<b>54</b>

Study sites are ordered from west to east. \* Included humpback dolphin mtDNA sequences and microsatellite genotypes published in Brown et al. (2014), plus one new sample from Cygnet Bay. ‡ Included snubfin dolphin samples (Roebuck Bay = 25; Cygnet Bay = 32) published in Brown et al. (2014). † The humpback dolphin sample collected at the Cambridge Gulf site was from a dolphin sampled in the Berkeley River, c. 50 km west of the mouth of the Cambridge Gulf.

##### 3.1.1 Snubfin dolphins

Analyses of mtDNA for snubfin dolphins revealed a haplotype diversity of 0.716 and seven unique haplotypes distributed among sampled individuals (Figure 3.1). Six of the total seven haplotypes were found at Cygnet Bay, and haplotypes found at the lesser-sampled sites of Yampi Sound and Prince Regent River were common among sites further west (Oh1 and Oh2).



**Figure 3.1.** Snubfin dolphin haplotypes as identified by a 382 base pair sequence of the mtDNA control region. Pie charts are scaled according to sample sizes, which are illustrated in parentheses next to the study site name. The number of individuals expressing each haplotype is indicated in the legend.

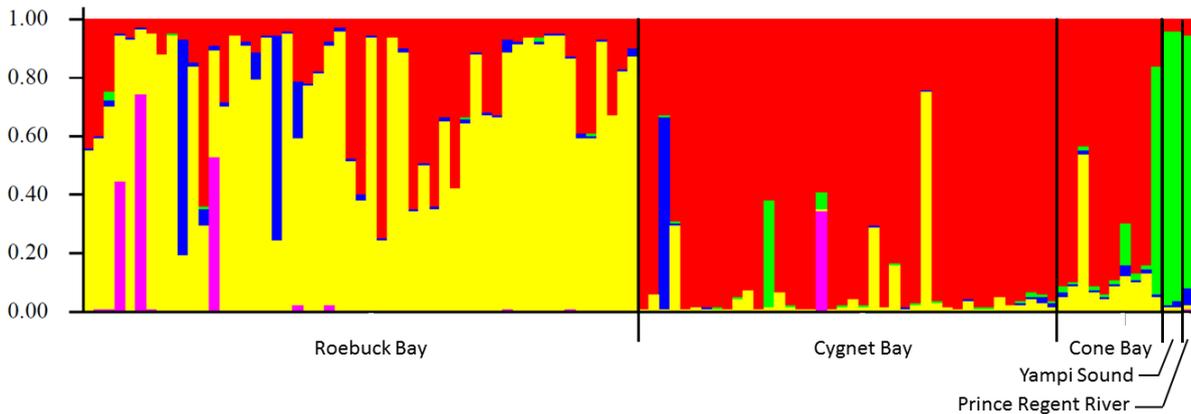
Analyses of genetic differentiation for snubfin dolphins were restricted to Roebuck, Cygnet and Cone Bay study sites due to the small sample sizes at Yampi Sound and Prince Regent River. Sampled snubfin dolphins at Roebuck Bay were significantly differentiated from those at Cygnet Bay and Cone Bay for mtDNA and microsatellites (Table 3.2) after Bonferroni correction. There was no significant differentiation between snubfin dolphins at Cygnet Bay and Cone Bay for mtDNA or microsatellites following Bonferroni correction.

**Table 3.2.** Microsatellite (above diagonal) and mtDNA (below diagonal) *F*<sub>st</sub> values and their significance levels for snubfin dolphins.

Site	Roebuck Bay	Cygnet Bay	Cone Bay
Roebuck Bay	-	0.070***	0.044***
Cygnet Bay	0.369***	-	0.025*
Cone Bay	0.322***	-0.052	-

\**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001. Note: when applying the Bonferroni correction of 0.05/(3\*11) = 0.0015, there is no longer significant differentiation between Cygnet and Cone Bay sites for microsatellites (bold value of 0.025).

All available snubfin dolphin samples were included in the STRUCTURE analysis. STRUCTURE assigned most individuals sampled at the same location to the same genetic cluster (Figure 3.2), with the greatest level of admixture apparent among animals sampled at Roebuck Bay. Both Ln*P*(*D*) and delta-*K* values suggested that the most likely number of clusters (*K*) was five; visual examination of the STRUCTURE plot revealed a majority of individuals to assign to one of two clusters: one dominating at Roebuck Bay and one dominating at both Cygnet Bay and Cone Bay (Figure 3.2). While sample sizes were small, there was strong partitioning of individuals sampled at Yampi Sound and the Prince Regent River, with one Cone Bay individual also assigned to the same cluster.



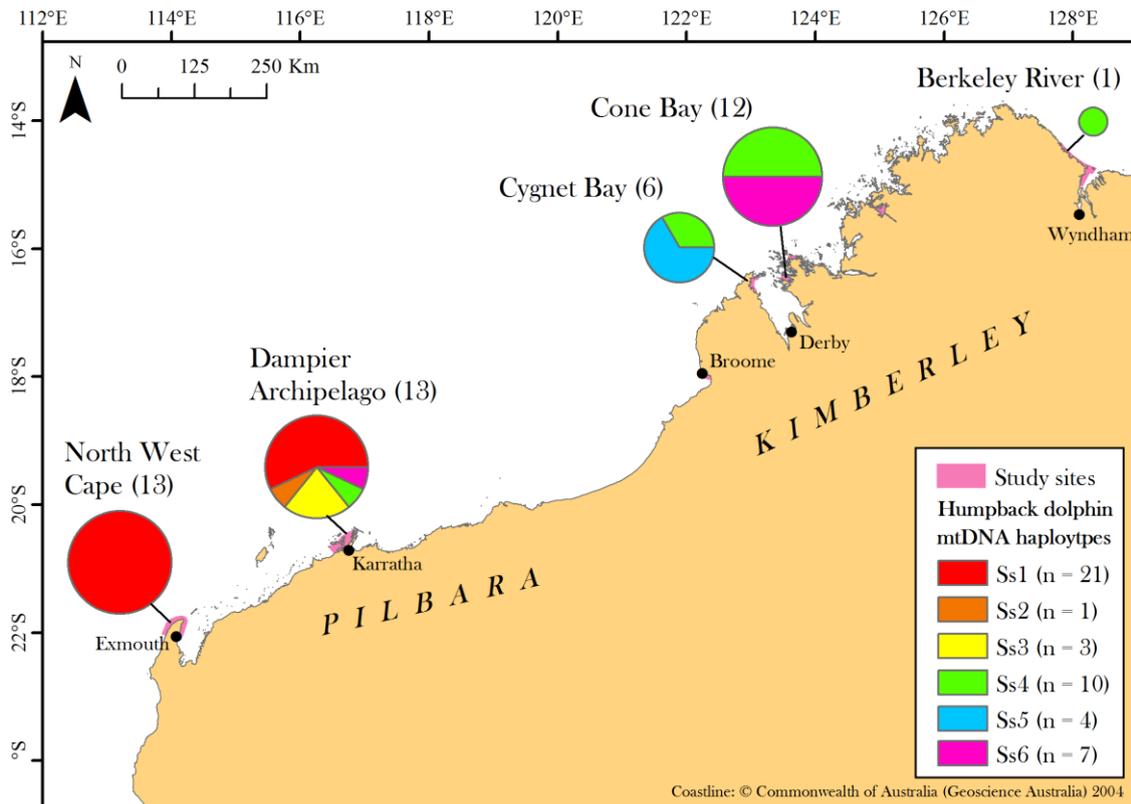
**Figure 3.2.** STRUCTURE plot for snubfin dolphins where K (number of clusters) = 5. Each bar on the x-axis corresponds to an individual, and the y-axis indicates that individual's proportion of membership to a genetic population/cluster.

Contemporary migration rates (i.e. within the last few generations) revealed an estimated proportion of 0.07 (95% CI 0.02-0.12) of snubfin dolphins in Roebuck Bay derived from Cygnet Bay, and 0.05 (95% CI 0.00-0.10) of Cygnet Bay individuals derived from Roebuck Bay. Sample sizes for snubfin dolphins at other sites were too small for contemporary migration rates to be calculated.

### 3.1.2 Humpback dolphins

Analyses of mtDNA for humpback dolphins at all sampled sites revealed a haplotype diversity of 0.718 and six unique haplotypes distributed among 45 sampled individuals. Considering only humpback dolphin samples from the Kimberley region, haplotype diversity was 0.667 with three unique haplotypes among 19 sampled individuals. Only two haplotypes were shared between the Pilbara and Kimberley regions, including single individuals each with haplotype Ss4 and Ss6 at the Dampier Archipelago (Figure 3.3). The haplotype of the single individual sampled at the far eastern site of the Berkeley River (Ss4) was shared by multiple individuals at the other two Kimberley sites and also one at the Dampier Archipelago. Haplotype Ss6 was unique to Cygnet Bay, where it was shared by four individuals.

Due to low numbers of humpback dolphins collected during the current study, microsatellite genotypes and mtDNA sequences from humpback dolphins sampled at the North West Cape (n=18) and Dampier Archipelago (n=17) reported in Brown et al. (2014) were included in analyses of genetic differentiation alongside those from Cygnet Bay (n=6) and Cone Bay (n=12). There was significant differentiation for humpback dolphins between all sampled sites, with the highest  $F_{st}$  values (> 0.200) reported between the eastern sites of Cone Bay, Cygnet Bay and those further west in the Pilbara (Table 3.3). By comparison, the level of differentiation between the two western sites of the North West Cape and Dampier Archipelago was relatively low ( $F_{st}$  = 0.045), and no longer significant following Bonferroni correction. We note that results for Cygnet Bay should be interpreted with caution due to the low sample size. Considering the genetic clustering results from STRUCTURE (see below), we also estimated the level of differentiation between two regionally pooled datasets: Pilbara (North West Cape and Dampier Archipelago); and, Kimberley (Cygnet Bay, Cone Bay, Berkeley River). Pilbara and Kimberley regions were significantly differentiated for both mtDNA and microsatellites.



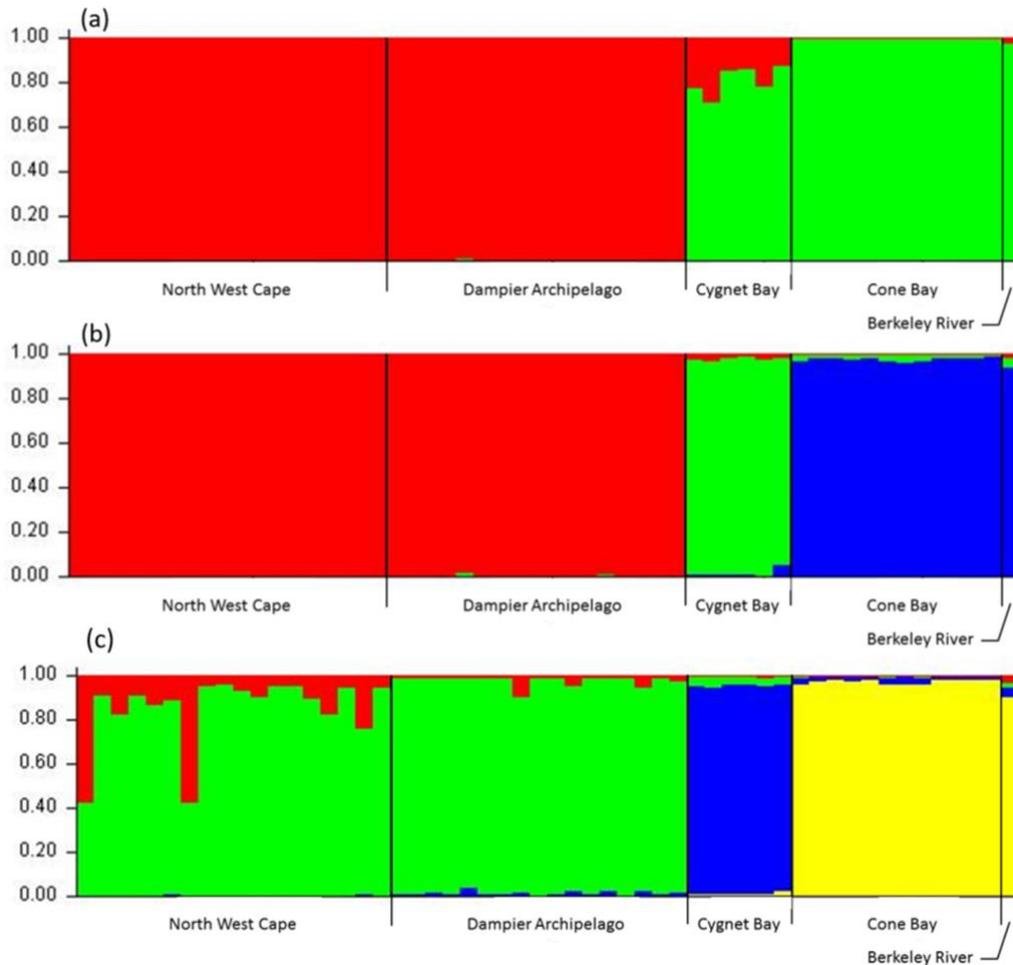
**Figure 3.3.** Humpback dolphin haplotypes as identified by a 391 base pair sequence of the mtDNA control region. Pie charts are scaled according to sample sizes, which are illustrated in parentheses next to the study site name. The number of individuals expressing each haplotype is indicated in the legend.

**Table 3.3.** Microsatellite (above diagonal) and mtDNA (below diagonal) *F<sub>st</sub>* values and their significance levels for humpback dolphins.

Site	NW Cape	Dampier Arch.	Cygnet Bay	Cone Bay	Pilbara	Kimberley
NW Cape	-	<b>0.045**</b>	(0.191***)	0.181***	-	-
Dampier Archipelago	0.167*	-	(0.208***)	0.211***	-	-
Cygnet Bay	(0.623***)	(0.300**)	-	(0.129***)	-	-
Cone Bay	0.825***	0.435***	(0.480**)	-	-	-
Pilbara	-	-	-	-	-	0.156***
Kimberley	-	-	-	-	0.413***	-

\**p* < 0.05; \*\**p* < 0.01; \*\*\**p* < 0.001. Values in parentheses should be interpreted with caution due to small sample size (*n*=6) at Cygnet Bay. Pilbara data include pooled samples from the North West Cape and Dampier Archipelago (*n*=26 for mtDNA; *n* = 35 for microsatellites); Kimberley data include pooled samples from Cygnet Bay, Cone Bay and the Berkeley River (*n*=19 for mtDNA; *n* = 19 for microsatellites). Note: when applying the Bonferroni correction of 0.05/(4\*13) = 0.0010, there is no longer significant differentiation between the North West Cape and Dampier Archipelago for microsatellites (bold value of 0.045).

All available humpback dolphin samples were included in the STRUCTURE analysis. Delta-K values suggested that the most likely number of clusters (*K*) was two, while Ln*P*(*D*) values plateaued above 2. At *K* = 2, the two genetic clusters separated animals sampled at the western two sites (North West Cape and Dampier Archipelago; 'Pilbara') and those sampled further east in the Kimberley Region (Cygnet Bay, Cone Bay, Berkeley River) (Figure 3.4). At *K* = 3, samples from Cygnet Bay formed a separate genetic cluster to those further east. At both *K* = 3 and *K* = 4, all but two individuals from the North West Cape and Dampier Archipelago were predominately assigned to the same genetic cluster, with *K* = 4 retaining the same three clusters of suggested as *K* = 3 but additional admixture of North West Cape animals indicated.



**Figure 3.4.** STRUCTURE plots for humpback dolphins where K (number of clusters) = 2 (a), K = 3 (b) and K = 4 (c). Each bar on the x-axis corresponds to an individual, and the y-axis indicates that individual's proportion of membership to a population/cluster.

Due to limited sample sizes for humpback dolphins from individual sites, and the STRUCTURE results suggesting two main genetic clusters of animals, sites were pooled and contemporary migration rates were calculated between Pilbara (North West Cape, Dampier Archipelago) and Kimberley (Cygnet Bay, Cone Bay, Berkeley River) regions. An estimated proportion of 0.01 (95% CI 0.00-0.03) of humpback dolphins in the Pilbara sampled derived from Kimberley samples sites, and 0.02 (95% CI 0.00-0.05) of individuals at Kimberley samples sites derived from the Pilbara sampled sites.

## 3.2 Relative abundance

### 3.2.1 Survey effort, sightings, group size and encounter rates

Snubfin and humpback dolphins were observed at all study sites in variable numbers (Table 3.4). Encounter rates were highest for snubfin dolphins in Cone Bay and the Prince Regent River (the river/inlet itself), and highest for humpback dolphins in the broader Prince Regent River area. However, the latter finding was strongly influenced by a single observation of a larger group of 10 humpback dolphins. For both species, mean group sizes were between 2-4, with single animals and pairs of individuals the most frequently observed. The largest groups observed were of comparable size between the two species, at 13 snubfin and 11 humpback dolphins.

**Table 3.4.** Sightings, group sizes and encounter rates for snubfin and humpback dolphins at study sites in the Kimberley region.

Study site	Survey effort (km)	Number of transect repeats / days of effort	Snubfin dolphins					Humpback dolphins				
			Number of group sightings (on effort only)	Group size: min-max	Group size: mean (SE) / mode	Total dolphins observed on survey effort	d/km (SE)	Number of group sightings (on effort only)	Group size: min-max	Group size: mean (SE) / mode	Total dolphins observed on survey effort	d/km (SE)
Cone Bay 2014	297	6 transects	20 (14)	1-13	3.8 (0.8) / 1	60	0.20 (0.08)	37 (12)	1-11	3.4 (0.4) / 2	21	0.07 (0.03)
Cone Bay 2015 <sup>†</sup>	na	na	11 (na)	1-12	3.6 (1.3) / 1	na	na	16 (na)	1-10	4.8 (0.8) / 2	na	na
Cone Bay 2014-2015	na	na	31 (na)	1-13	3.7 (0.7) / 1	na	na	53 (na)	1-11	3.8 (0.4) / 2	na	na
Cambridge Gulf	476	9 days	22 (17)	1-8	1.9 (0.4) / 1	34	0.07 (0.02)	18 (15)	1-7	2.7 (0.4) / 2	42	0.09 (0.03)
Prince Regent R. Area <sup>**</sup>	323	6 days	16 (16)	1-4	2.2 (0.3) / 2	35	0.11 (0.03)	10 (9)	1-10	3.3 (0.9) / 2	31	0.15 (0.05)
Prince Regent R. (river only)	147	4 days	12 (12)	1-4	2.3 (0.3) / 2	28	0.19 (0.01)	5 (5)	1-6	3.4 (0.7) / 2	17	0.11 (0.04)

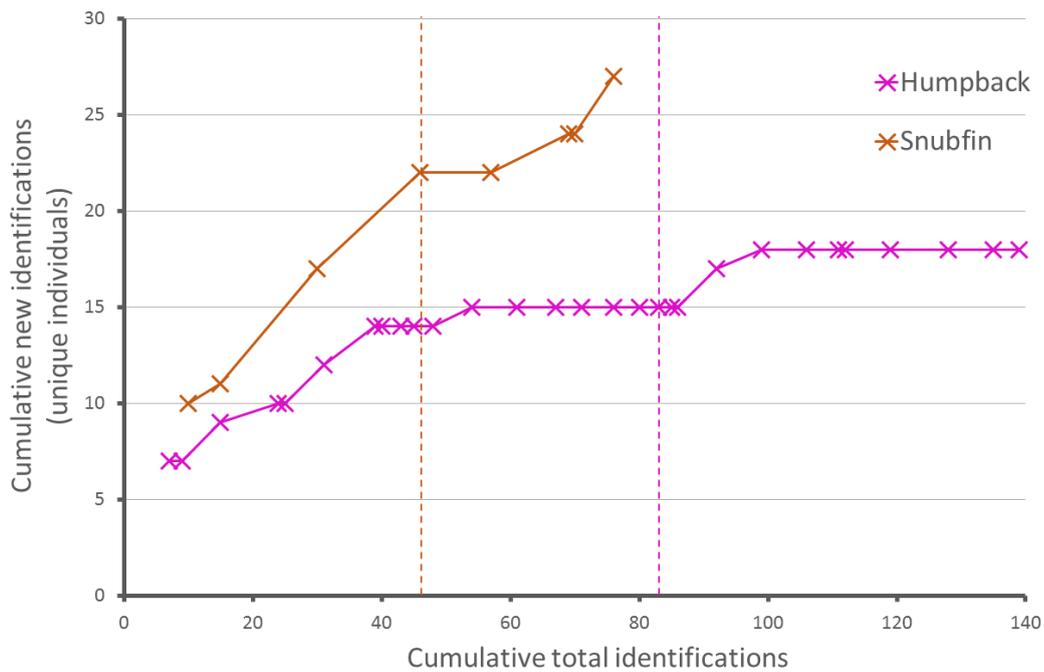
<sup>†</sup> Sightings recorded in Cone Bay in 2015 were opportunistic during acoustic data collection; no structured survey effort was conducted during this period.

<sup>\*\*</sup> Including Rothsay Water, Munster Water, Saint George Basin and the Prince Regent River.

Maps of the sighting locations and group sizes are provided for all study sites in Appendix 2. While snubfin and humpback dolphins were both observed across much of Cone Bay, snubfin dolphins were more frequently observed in the inner reaches of the bay closer to mangrove areas. Humpback dolphins were regularly observed in the proximity of the barramundi fish farm adjacent to Turtle Island in Cone Bay, including among the sea cages. In the Prince Regent River area, the river/inlet itself was where most snubfin dolphin sightings occurred, although effort was concentrated in the area. Snubfin and humpback dolphins were observed through most areas surveyed in the Cambridge Gulf and adjacent coastal waters, including river mouth, open coast and shallow ( $\leq 30$  m) habitats further offshore.

### 3.2.2 Photo-identification

Photo-ID was successful in Cone Bay, with images of acceptable quality obtained for 95% of humpback and 67% of snubfin dolphins (excluding calves) encountered during the 2014 trip. A total of 27 distinctive snubfin and 18 humpback dolphin individuals were photo-identified over the 2014 and 2015 data collection periods in Cone Bay. The majority of these individuals, 22 snubfin and 15 humpback, were identified in 2014, which represented a greater field effort. A cumulative identification plot (Figure 3.5) revealed a plateau in the rate of new humpback dolphin identifications, suggesting that the 18 distinctive individuals represent the majority of animals using the area. While a greater number of unique individual snubfin dolphins were identified than humpbacks, there were fewer identifications overall. Snubfins also showed no evidence of a plateau in the rate of new identifications, suggesting that the total 27 distinctive individuals may represent only a subset of the total individuals using the area. Of the 15 humpback dolphins photo-identified in 2014, 10 (67%) were also observed in 2015, while 13 (59%) of the 22 snubfin dolphins identified in 2014 were also observed in 2015. Data on the relative abundance of dolphins at Cone Bay in 2014 is published in Brown et al. (2016).



**Figure 3.5.** Cumulative identification plot for humpback and snubfin dolphins at Cone Bay from 2014-2015. The plot illustrates how the cumulative number of new identifications (y-axis) relates to the cumulative total number of identifications (x-axis, including re-sights). Vertical dashed bars illustrate the last incident of identifications in 2014 for each species.

The dolphins encountered in the Cambridge Gulf and surrounds proved boat-shy and difficult to approach. Acceptable quality photo-ID data was obtained for only 15% of snubfin and 40% of humpback dolphins encountered. Where suitable quality images were obtained, a total of 12 humpback dolphin individuals and six snubfin dolphin individuals were identified from unique marks on their dorsal fins. A humpback dolphin mother and calf were photo-identified among a group of seven animals on 09 October off the open coast to the west of

the Cambridge Gulf, close to Buckle Head. This same mother and calf were then observed off the mouth of the Helby River, within the Cambridge Gulf, on 12 October, a location approximately 52 km distant from the previous sighting.

Moderate success in collecting photo-ID data was achieved in the Prince Regent River area: 57% of snubfin and 71% of humpback dolphins sighted were successfully photographed to a suitable standard for identifying individuals based on distinct notches on their dorsal fins. For the Rothsay Water to Prince Regent River as a whole, nine distinctive snubfin and 13 distinctive humpback individuals were photo-identified. These numbers exclude dependent calves, which are not usually distinctively marked. In the Prince Regent River alone, eight distinctive snubfin and four distinctive humpback individuals were photo-identified.

### 3.3 Passive Acoustic Monitoring

#### 3.3.1 Soundscape monitoring

In Roebuck Bay, approximately 940 h of acoustic recordings were collected over a total of 39 days during the two deployments. Examples of the soundscape are depicted as a series of long-term spectrograms from the July 2014 dataset in Appendix 4. Over the recording period, snapping shrimp were the most prevalent sound source in Roebuck Bay. Sounds from snapping shrimp were high-amplitude, impulsive signals observed periodically. However, the most prominent sound source was evening fish choruses. Fish choruses were of various types, here referred to as call Types I to VI (Figure 3.6). Choruses were most pronounced at dusk. Choruses of call Type I had energy between frequencies of approximately 200 and 1000 Hz. Calls of Type I produced by individual fish were also recorded, but mainly during the day. Call Type II was tonal at approximately 100 Hz, while call Type III was a series of impulsive signals around 300 Hz. Call Type IV had energy between approximately 100 and 500 Hz, and call Type V between approximately 50 and 300 Hz. Finally, Call Type VI began at approximately 50 Hz and increased to 200 Hz.

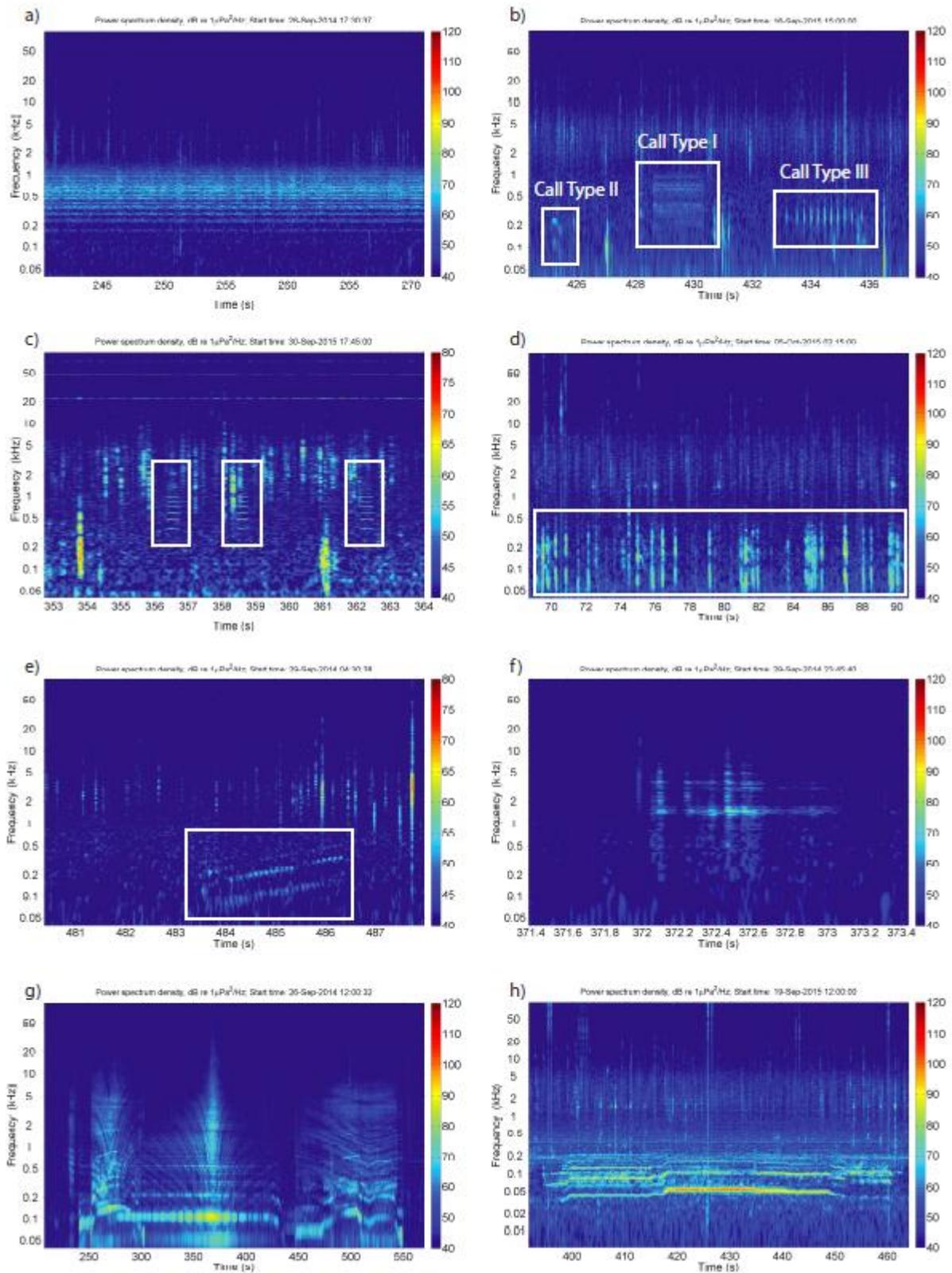
Noise from the movement of nearby cyclone mooring chains caused sporadic, intense sounds around 1.5 kHz. Broadband noise from vessel propeller cavitation was relatively infrequent, but at times intense. Vessel narrowband tones from engine and propeller rotation were also visible in spectrograms (Figure 3.6).

There was temporal variability in broadband noise levels both on a daily basis and between the two sampling periods of July and September/October (Figure 3.7). In July, broadband levels were 87 dB re 1  $\mu$ Pa rms and, in September-October, they were 84 dB re 1  $\mu$ Pa rms (10 Hz – 11 kHz). The higher levels in July were associated with increased vessel noise.

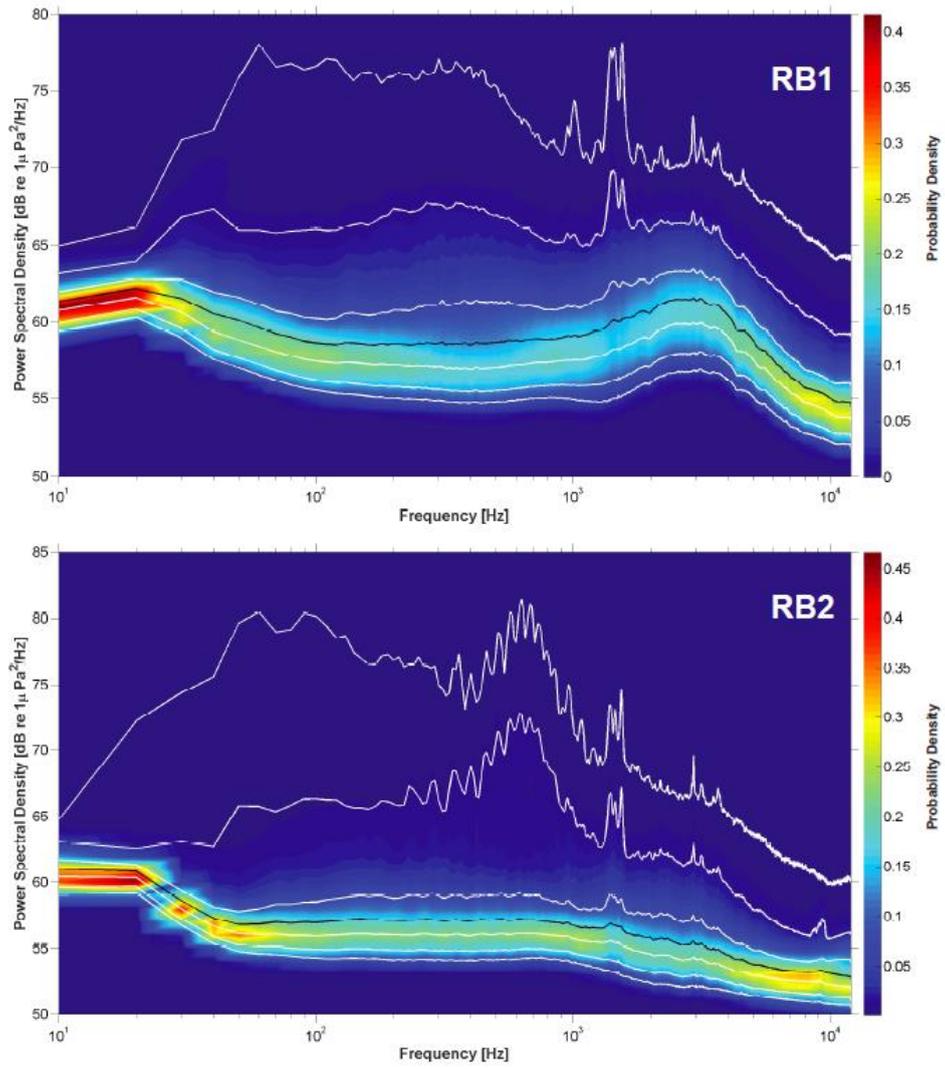
Fish choruses (particularly fish call Type I) were more intense in September-October than those in July, as was sound from snapping shrimp. There were peaks in underwater noise at 1.5 kHz during both July and September-October sampling periods, which were most likely noise associated with the movement of the cyclone moorings nearby.

Over the diurnal cycle, noise levels were generally highest in the early evening, corresponding with times fish choruses were most prevalent (Appendix 4). Evening fish chorus, peaking at around 600-700 Hz, increased noise levels considerably.

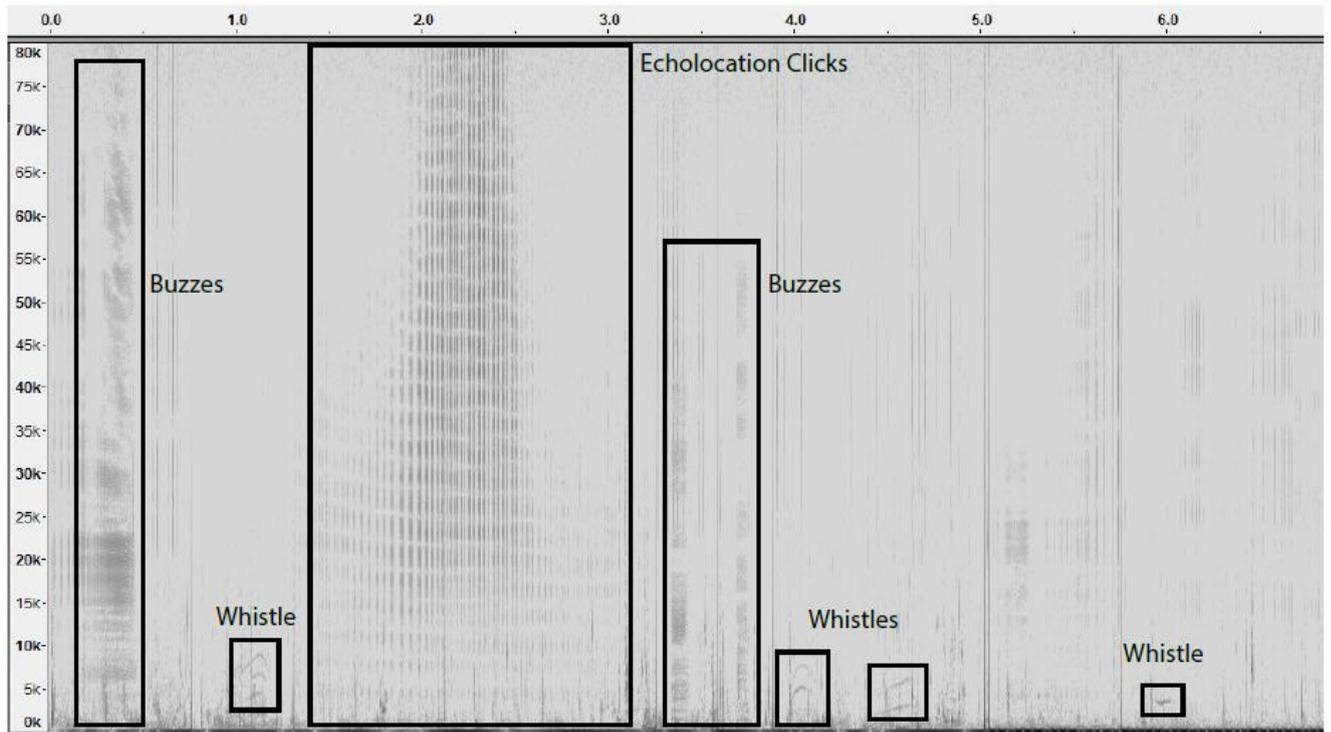
While present, sounds produced by dolphins were not prominent in long-term spectrograms because they are transient, short duration signals. Finer-scale inspection of the acoustic data was required to locate these signals and echolocation clicks, buzzes, and whistles produced by dolphins were present within recordings (Figure 3.8).



**Figure 3.6.** Examples of sound sources recorded in Roebuck Bay: (a and b) fish call Type I, (b) fish call Types II and III, (c) fish call Type IV, (d) fish call Type V, (e) fish call Type VI, (f) mooring noise, and (g and h) vessel noise.



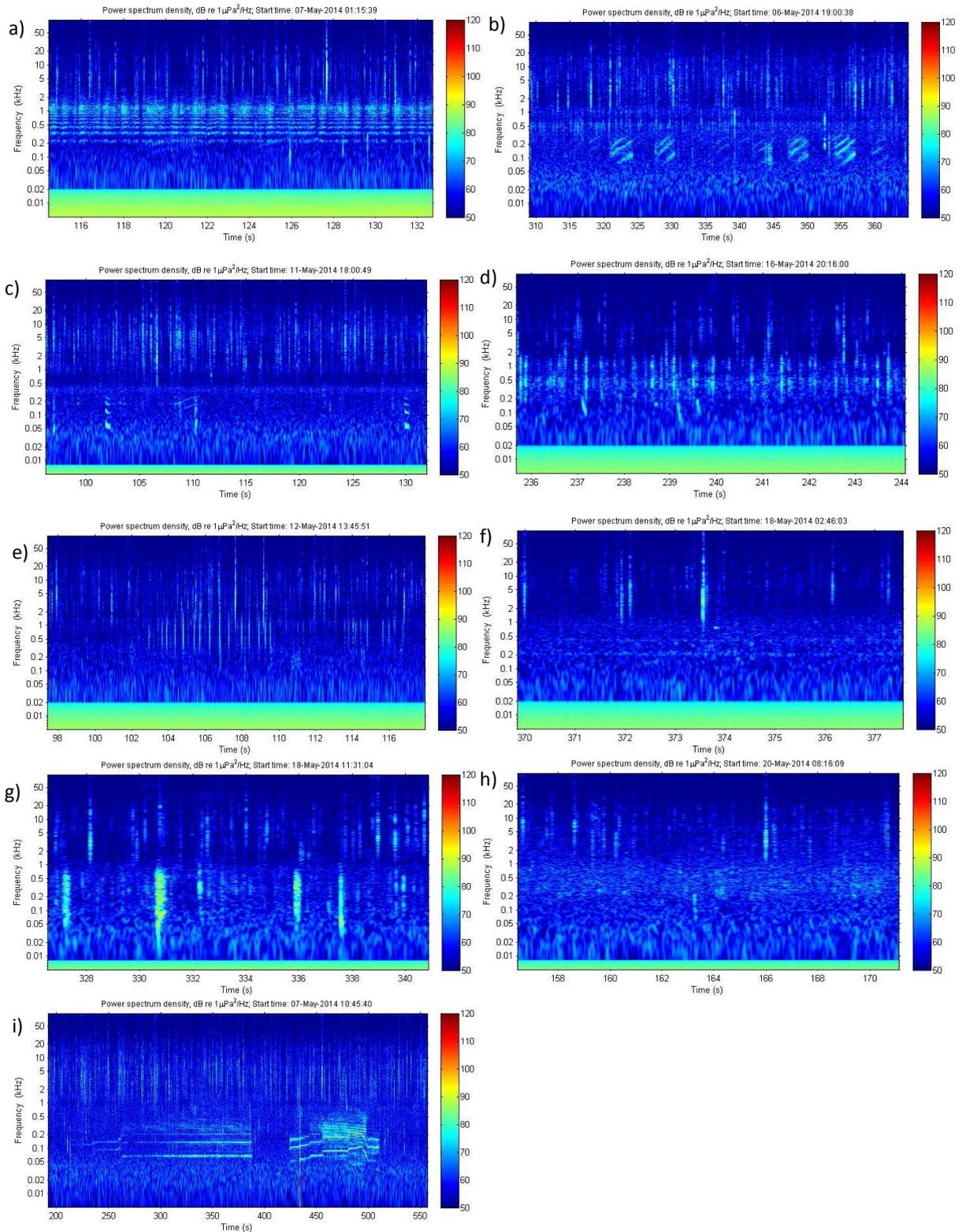
**Figure 3.7.** Power Spectrum Density (PSD) percentile plots of the soundscape in Roebuck Bay in July (RB1) and September-October (RB2).



**Figure 3.8.** Broadband echolocation clicks, pulsed ‘buzz’ sounds, and tonal whistles recorded in the presence of snubfin dolphins.

### *Cygnets Bay*

At Cygnets Bay, a total of approximately 305 h of acoustic recordings were made during 20 days in May 2014. A representation of the Cygnets Bay soundscape is depicted as a series of long-term spectrograms in Appendix 5. As in Roebuck Bay, snapping shrimp were the most prevalent sound source, occurring throughout the recording period. However, again the most prominent sound source was evening fish choruses. In Cygnets Bay, evening fish choruses were generally more intense and occurred more regularly than in Roebuck Bay (every evening throughout the recording period; Appendix 5). The predominant fish chorus was call Type I, also recorded in Roebuck Bay. In addition, fish call Types II and VI (previously described as individual calls in Roebuck Bay) were present as choruses in Cygnets Bay (Figure 3.9). Choruses were most pronounced between dusk and dawn. Calls not prevalent in Roebuck Bay but recorded in Cygnets Bay included call Type VII, which was impulsive, with energy between approximately 200 Hz and 1 kHz.



**Figure 3.9.** Examples of sound sources recorded in Cygnet Bay: (a) fish call Type I, (b) fish call Types VI, (c) fish call Type II, (d) fish call Type VII, (e) unknown knocking, (f) unknown background noise, (g) unknown 'bump', (g) (h) unknown background noise, and (i) vessel noise.

Noise from the movement of the acoustic receiver mooring caused frequent intense noise and background lower level broadband noise (Figure 3.9). Broadband noise also included sound from various unknown sources, ranging in frequencies from 20 Hz to above 50 kHz (Figure 3.9). In addition, noise from vessel propeller cavitation, and narrowband tones from engine and propeller rotation, occurred intermittently and at times were intense (Figure 3.9).

As in Roebuck Bay, dolphin sounds were not prominent in long-term spectrograms and required finer-scale inspection of the acoustic data to locate these signals. There was temporal variability in broadband noise levels throughout the day (Appendix 4) and over the recording period. Overall, noise levels were higher between dusk and dawn due to fish choruses. Snapping shrimp noise was most intense in early May. Mooring and vessel noises caused peaks in underwater noise below 1 kHz. Peaks between 600 and 700 Hz, corresponding to frequencies of fish choruses, increased noise levels considerably.

### 3.3.2 Vocal detections in relation to dolphin occurrence

#### *Roebuck Bay*

This section focuses on vocal detections in relation to dolphin sightings. An analysis of the relative abundance of dolphins in Roebuck Bay (as was presented for other sites in the sections above) is beyond the scope of the current project, but was addressed in Brown (2016) and will be the focus of future work. In this report, a brief description of visual observations is made to provide context for presenting the association between acoustic and visual detections.

A total of 16 vessel surveys were conducted, 13 of which were dedicated surveys with effort recorded. During these 16 surveys, a total of 375 individual dolphins were sighted in 111 groups (Table 3.5). Of these, 342 were snubfin, 16 were humpback, and 17 were bottlenose dolphins. Group sightings were made every day, ranging from one to 19 groups sighted in a day. On-survey effort ranged from 5.2 - 48.6km/day.

**Table 3.5.** Dolphin sightings, group sizes and encounter rates for snubfin, humpback and bottlenose dolphins during vessel surveys and non-dedicated survey days (\*; including logger deployments) in Roebuck Bay in July 2014.

Survey Date	On-Effort Survey (km)	Snubfin dolphins					Humpback dolphins			Bottlenose dolphins		
		No of group sightings	Group size: min-max	Group size: mean (SE) / mode	Total dolphins observed on survey effort	d/km	No of group sightings	Total dolphins observed on survey effort	d/km	No of group sightings	Total dolphins observed on survey effort	d/km
4/7/2014*	NA	1	7-7	7.0	7	NA	0	0	0	0	0	0
6/7/2014*	NA	5	1-5	3.2 (0.8) / 5	16	NA	0	0	0	0	0	0
7/7/2014	7.7	4	1-3	1.5 (0.5) / 1	6	0.8	1	5	0.6	0	0	0
8/7/2014	48.6	6	1-4	1.8 (0.5) / 1	11	0.2	0	0	0	0	0	0
10/7/2014	7.7	3	2-3	2.7 (0.3) / 3	8	1.0	0	0	0	0	0	0
11/7/2014	21.6	6	1-3	1.5 (0.3) / 1	9	0.4	0	0	0	1	4	0.2
12/7/2014	7.8	9	1-11	4.1 (1.1) / 1	37	4.7	0	0	0	0	0	0
17/7/2014	5.2	5	1-7	3.8 (1.0) / 3	19	3.7	0	0	0	0	0	0
18/7/2014	32.8	5	2-14	5.8 (2.2) / 2	29	0.9	0	0	0	0	0	0
19/7/2014	34.2	13	1-5	2.5 (0.4) / 1	33	1.0	0	0	0	0	0	0
21/7/2014	20.2	17	1-15	4.2 (1.0) / 3	72	3.6	0	0	0	1	6	0.3
22/7/2014	12.6	7	2-16	5.9 (1.9) / 2	41	3.3	0	0	0	0	0	0
23/7/2014	35.5	6	1-12	3.3 (1.8) / 1	20	0.6	0	0	0	0	0	0
27/7/2014	24.9	8	1-5	3.3 (0.5) / 3	26	1.0	0	0	0	0	0	0
28/7/2014	21.7	8	1-3	1.6 (0.3) / 1	13	0.6	0	0	0	0	0	0

The highest frequency of dolphin sightings was in the north of the survey area (Figure A5.1), with group sizes ranging from one to 16 individuals. Bottlenose and humpback dolphin groups were rarely seen, and were sighted toward the southern end of the transects or in transit near the middle of the bay (Figure A3.1).

Transects within Stratum 2 (the smaller area where the acoustic receiver was located) were surveyed 12 to 13 times, while transects in the larger Stratum 1 were surveyed one to five times (depending upon the transect). Stratum 2 received a higher level of effort for the purpose of collecting data to compare visual and acoustic detections.

Acoustic measurements from the hand-held hydrophone deployed off the survey vessel during dolphin encounters included a total of 125.3 min in 20 recordings. Recordings ranged from approximately 3 to 10 min with a mean of 5.9 min. One recording was made during an encounter with a humpback dolphin group, two during encounters with bottlenose dolphin groups, 16 during snubfin dolphin encounters, and one during a mixed species group of bottlenose and snubfin dolphins. Of the 20 recordings, 17 contained dolphin sounds. Thus, 85% of all recordings detected dolphins within visual detection range.

During snubfin encounters 94% of recordings contained dolphin sounds. Two of the three bottlenose dolphin encounters contained dolphin sounds. The humpback dolphin encounter contained no dolphin sounds. Dolphin sounds were recorded in the mixed species encounter.

Overall, sounds detected during snubfin dolphin encounters were dominated by broadband echolocation click trains (55%), followed closely by burst-pulse sounds (43%). Few whistles were detected during snubfin dolphin encounters (2%). Sounds recorded during the encounter with bottlenose dolphins included echolocation click trains and whistles during one encounter, and only clicks in the second encounter. The single recording made during the mixed species group of snubfin and bottlenose dolphins contained only clicks.

**Table 3.6.** Hand-held acoustic recordings obtained during vessel surveys in Roebuck Bay, July 2014.

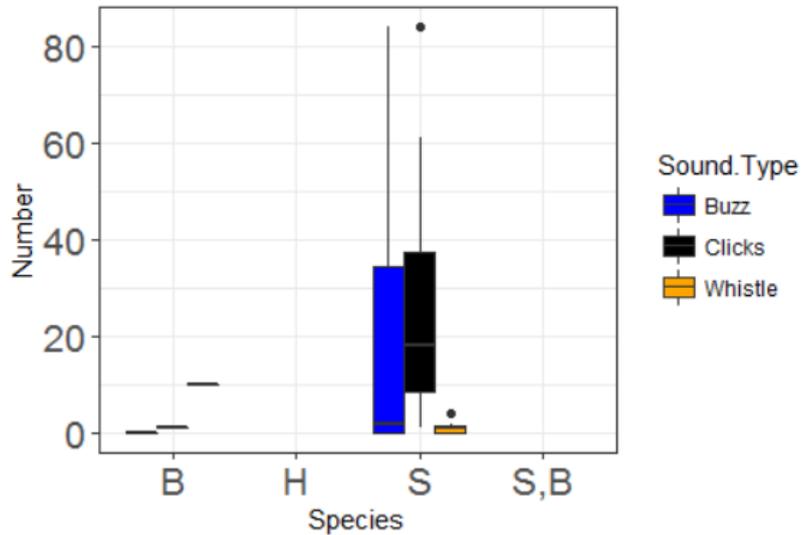
Date	Acoustic Rec	Recording duration	Dolphin sounds	Species	Estimated group size	Clicks	Buzzes	Whistles
7/07/2014	T0101_1	05:56	N	H	5	-	-	-
11/07/2014	T0106_1	05:01	Y	S	1	2	0	2
11/07/2014	T0109_1	05:19	Y	B	4	1	0	10
11/07/2014	T0113_1	05:34	N	S	3	-	-	-
12/07/2014	T0116_1	04:28	Y	S	8-14	22	48	0
12/07/2014	T0117_1	05:28	Y	S	8-14	21	74	0
12/07/2014	T0119_1	06:03	Y	S	6	9	2	1
17/07/2014	T0130_1	07:00	Y	S	4-5	59	1	0
19/07/2014	T0149_1	03:12	Y	S	2-3	1	0	0
19/07/2014	T0153_1	05:52	Y	S	1	8	0	1
19/07/2014	T0154_1	10:00	Y	S	4	84	4	4
21/07/2014	T0159_1	05:21	Y	S	7-8	18	34	1
21/07/2014	T0160_1	05:03	N	B	6-7	1	-	-
21/07/2014	T0162_1	07:55	Y	S	5-7	2	1	1
21/07/2014	T0168_1	05:37	Y	S	10-14	16	26	4
21/07/2014	T0169_1	05:46	N	S	3	-	-	-
22/07/2014	T0183_1	08:29	N	S,B	1	-	-	-
23/07/2014	T0195_1	05:08	Y	S	4-13	16	35	0
27/07/2014	T0198_1	03:03	Y	S	5-6	43	0	0
27/07/2014	T0202_1	10:02	Y	S	8-9	61	84	2
28/07/2014	T0208_1	03:13	Y	S	10-14	32	0	0

Species include: snubfin (S), humpback (H) and bottlenose (B) dolphins.

When click trains were present during snubfin dolphin encounters, the number detected per recording ranged from one to 84 (Figure 3.12). More than 15 click trains were detected during 13 recordings in the presence of snubfin dolphins, one contained nine detections, and three had fewer than two click trains detected. When

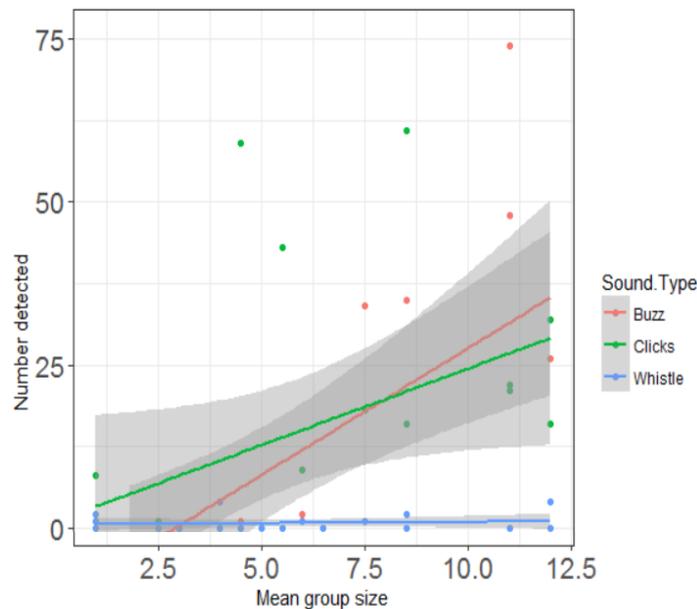
buzzes were present during snubfin dolphin encounters, the number ranged from one to 84 per recording. Five of the 17 recordings in their presence contained fewer than two detected. When whistles were present, fewer than four were detected in all recordings.

The recording with whistles present during a bottlenose dolphin encounter had comparatively more whistles (10). However, the number of click trains detected per recording (when these sounds were present) was substantially lower (1 per recording).



**Figure 3.12.** Distribution of the number of sounds (buzzes, click trains, and whistles) detected per hand-held hydrophone recording during snubfin (S), bottlenose (B), and humpback dolphin (H), and mixed group (snubfin and bottlenose) encounters in Roebuck Bay in 2014.

The number of click trains and buzzes detected per recording was greater when large group sizes were sighted in the survey area. However, there was high variability associated with this trend (Figure 3.13) and these were not significantly correlated.



**Figure 3.13.** Correlation between click trains, buzzes, and whistles detected per recording and dolphin group size (linear model smoothing with 95% confident intervals applied).

During vessel surveys within Stratum 2, the acoustic receiver (logger) on the sea bed detected the presence of dolphins during 67% of the 12 surveys conducted (Table 3.7). Dolphins were detected visually during all of these surveys, and included a total of 109 individuals in 32 snubfin dolphin groups. Three of the four groups that were visually detected but not acoustically detected were composed of single individuals.

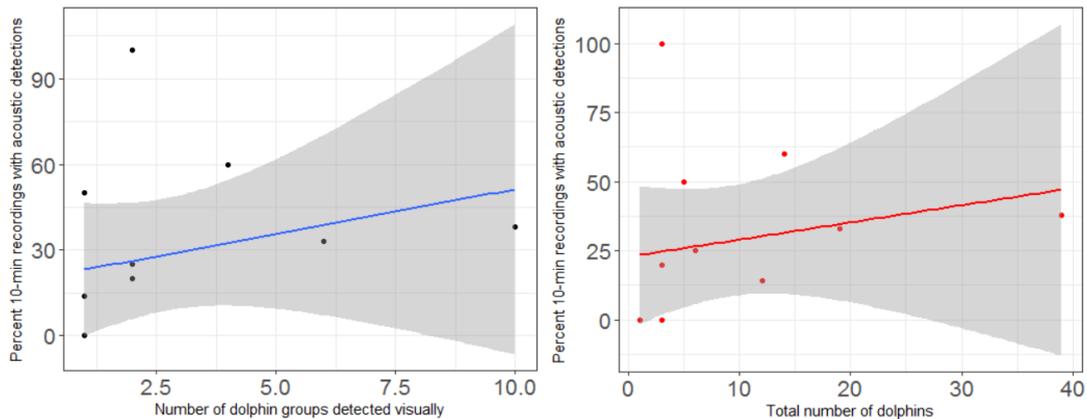
Echolocation clicks dominated the detections, with whistles and buzzes detected during two surveys. For vessel surveys with visual and acoustic detections, the percent of 10-min recordings containing detections ranged from 14 to 100%.

**Table 3.7.** Acoustic and visual detections during Stratum 2 vessel surveys in Roebuck Bay, July 2014.

Date	Number of groups detected visually	Group sizes (best estimate)	Detected acoustically (Y/N)	Percent of recordings with sounds
7/07/2014	1	3	No	0
7/07/2014	1	1	No	0
8/07/2014	1	1	No	0
10/07/2014	1	1	No	0
11/07/2014	2	1,2	Yes <sup>w</sup>	20
12/07/2014	4	1,3,4,6	Yes <sup>e</sup>	60
19/07/2014	2	3,3	Yes <sup>e</sup>	25
21/07/2014	10	2,3,15,6,2,3,2,2,3,3	Yes <sup>e,b</sup>	38
22/07/2014	1	5	Yes <sup>e</sup>	50
23/07/2014	1	12	Yes <sup>e</sup>	14
27/07/2014	6	1,5,3,4,3,3	Yes <sup>e</sup>	33
28/07/2014	2	2,1	Yes <sup>e</sup>	100

w=whistle, b=buzz, e=echolocation clicks

There was high variability in the percent of 10-min recordings containing acoustic detections and the total number of dolphins and dolphin groups detected during surveys in Stratum 2 (Figure 3.14).



**Figure 3.14.** Correlation between percent of 10-min acoustic recordings containing detections and number of dolphin groups (a), and total number of dolphin (b) detected visually during Stratum 2 surveys (linear model smoothing with 95% confident intervals applied).

### 3.3.3 Dolphin acoustic repertoire, behavioural context and geographic variation

Due to several of the field locations having overarching aims and objectives, the data relating to acoustic repertoire and vocalization types exhibited during different behaviours are presented in relation to the aims, and the data combined rather than reporting on individual field sites.

In the literature, more is known regarding humpback dolphin acoustics compared to snubfin dolphins; consequently, greater effort was expended obtaining acoustic samples of snubfin dolphins. Each of the

fieldwork locations had varying degrees of species composition and abundance and therefore the focus of the aims were shifted based on this, with sample sizes reflected accordingly (Table 3.7). Cygnet Bay typically had a higher abundance of snubfin dolphins with larger group sizes than humpback dolphins. Humpback dolphins in Cygnet Bay were more prone to exhibiting cryptic behaviour compared to snubfin dolphins, which is demonstrated by less surface active behaviour and less noticeable surfacing behaviour. For these reasons it was harder to obtain acoustic samples from humpback dolphins in Cygnet Bay compared to the other two field sites. From an acoustics perspective, Roebuck Bay and Cone Bay were more reliable in obtaining acoustic recordings of snubfin and humpback dolphins respectively.

**Table 3.7.** The group sizes, number of individual dolphins and duration of acoustic recordings obtained for humpback and snubfin dolphins during three fieldtrips in the Kimberley.

Survey site	Cygnet Bay (2014)		Roebuck Bay (2014)		Cone Bay (2015)	
	Humpback	Snubfin	Humpback	Snubfin	Humpback	Snubfin
Number of dolphin groups sampled	5	12	0	78	30	6
Number of individual dolphins sampled	21	97*	0	153*	130*	25
Moored recorder sampling (hrs:min)	305:00 (CMST)		55:58 (SoundTrap)		103:16 (SoundTrap)	
Portable acoustic recordings (hrs:min)	2:49		N/A		3:56	

\*NOTE: Photo-identification was not always taken and therefore there is likely repeated sampling of the same individuals.

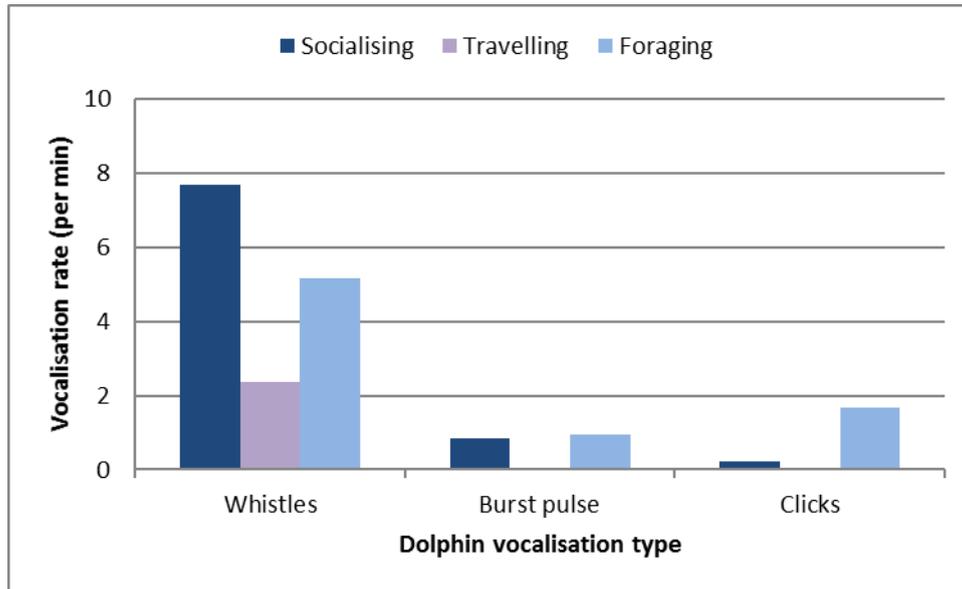
In relation to behaviour, there were relatively similar percentages of behavioural states exhibited by both snubfin and humpback dolphins across the three Kimberley field sites, with travelling and foraging being the predominant behaviours exhibited (Table 3.8). Cygnet Bay was an exception for foraging behaviour, however, showing a higher prevalence for socialising behaviour by both snubfin and humpback dolphins. Typically, the types of dolphin vocalisations that are produced vary with behaviour and generally whistles and burst pulses are more often produced during socialising and milling behaviour whereas broadband clicks are produced during foraging behaviour. Consequently, the choice of field site and the type of behaviour that can be observed is an extremely important consideration in passive acoustic monitoring.

**Table 3.8.** A breakdown of the behaviours exhibited by humpback and snubfin dolphins during acoustic sampling in the Kimberley.

Survey site	Cygnet Bay (2014)		Roebuck Bay (2014)		Cone Bay (2015)		
	Humpback	Snubfin	Humpback	Snubfin	Humpback	Snubfin	
Behaviour	Foraging	3.1%	12.5%	N/A	20.8%	42.9%	33.3%
	Socialising	35.9%	34.4%	N/A	5.2%	16.7%	33.3%
	Milling	0%	2.1%	N/A	1.0%	7.1%	0%
	Travelling	60.9%	51.0%	N/A	72.9%	33.3%	33.3%

A consistent pattern was found in the vocalisation rates of humpback dolphins for the three different vocalisation types for different behaviours. Dolphins were more vocal per unit of time whilst socialising, compared to when foraging or travelling. Whilst travelling, dolphins were the least vocal: no burst pulses or clicks were identified, and dolphins producing the least number of whistles per unit of time compared to when socializing and foraging (Fig.3.12). Whistles were the most common vocalisation type when socializing, although were also common during foraging behaviour. Burst pulse sounds and broadband clicks had relatively similar vocalization rates, lower than whistles, although broad-band clicks were predominantly used while foraging (Fig. 3.12). At this stage, vocalization rates have only been calculated for humpback dolphins because

the mobile recordings consisted largely of humpback dolphin recordings and a more efficient detection algorithm is needed to process some remaining moored acoustic recorder recordings for snubfin dolphins.



**Figure 3.12.** Vocalisation rates calculated for humpback dolphin whistles, burst pulse sounds and broad band clicks recorded during each behaviour

In relation to PAM, most research has focused on the detection of either whistles and clicks because they are typically easier to develop detection algorithms for compared to burst pulses. Currently, another study is investigating the parameters of clicks for both snubfin and humpback dolphins to determine if there are any statistical differences between the species to be an effective basis for PAM. Consequently, the focus here is on characterizing whistles within the acoustic repertoire of these species. Acoustic recordings from the Kimberley fieldwork have contributed to obtaining new examples of dolphin whistles to the acoustic repertoire of both humpback and snubfin dolphins. However, currently there has only been a qualitative comparison of the whistles from the Kimberley data to the existing acoustic repertoire database and therefore the data should be considered as preliminary findings. Furthermore, not all of the acoustic recordings have been processed and analysed for dolphin vocalisations. To date, the mobile recordings have been processed, although the acoustic recordings from the moored acoustic recorders still need to be processed. Development of a more effective algorithm to undertake automatic processing and detection of snubfin and humpback dolphins within the large amount of acoustic data is still needed to facilitate this process. Manual detection of acoustic recordings has been undertaken on the mobile recordings to aid in the development of a more effective algorithm.

Considering there is a greater amount of literature on humpback dolphin acoustics, there were fewer new whistle types found from the Kimberley data compared to snubfin dolphins. There are two new humpback dolphin whistle types that have so far been found from the Kimberley acoustic data that are not represented within the literature (Table 3.9; whistle 18,19). Of interest, there are a number of whistles that demonstrate ultrasonic frequency harmonics (up to approximately 70kHz) that have not been well documented in the literature due to most recording equipment that has previously been used being limited to approximately 22kHz which near the top end of human hearing range. Further, quantitative analyses will be done on the data once final processing of all the available data has been undertaken.

**Table 3.9.** A list of the acoustic repertoire of humpback dolphins in Australia, including whistles obtained during Kimberley fieldwork.

ID	Name	Min (kHz)	Max (kHz)	Dur (ms)	N
Wh1	Hook	8	13.8	140	87
Wh2	Short hook	7.4	9.4	110	67
Wh3	Slope	11.3	12.7	960	6
Wh4	Vase	6.9	17.1	260	153
Wh5	Uphill	9.7	12.4	73	21
Wh6	Hanger	3.5	8.1	210	0
Wh7	Slant Roof	8	13.4	880	0
Wh8	Chirp	3.2	4.3	890	2
Wh9	Snakes	4.4	13.9	820	38
Wh10	Test Tube	5.1	6.1	170	103
Wh11	Chinese	6.9	17	260	12
Wh12	Spike	5.4	7.2	119	11
Wh13	Squiggle	10.8	13.1	366	6
Wh14	Mountain	4.2	13.3	498	5
Wh15	Serpentine	3.7	11.6	466	14
Wh16	The Line	7.5	9.2	565	12
Wh17	The Tick	8.4	11.7	950	10
Wh18	The Pick	6.9	17	260	3
Wh19	Diagonal	3.8	7.8	270	2

Generally, there was less variation in the range of snubfin dolphin whistles compared to humpback dolphins and there also appears to be fewer different whistle types in the acoustic repertoire of snubfin dolphins. There is one new snubfin dolphin whistle type that has so far been found from the Kimberley acoustic data that are not represented within the literature (Table 3.10; whistle 7). Similar to humpback dolphins there were a large number of whistles with harmonics, although the number of harmonics were fewer. The mean minimum frequency for humpback dolphins was 6.5kHz compared to 3.5Khz for snubfin dolphins, suggesting that potentially snubfin dolphins may produce whistles of lower frequency than humpback dolphins.

**Table 3.10.** A list of the acoustic repertoire of snubfin dolphins in Australia, including whistles obtained during Kimberley fieldwork.

ID	Name	Min (kHz)	Max (kHz)	Dur (ms)	N
Wh1	Slope	3.3	5.6	271	21
Wh2	Diagonal	4.1	8.0	285	6
Wh3	U	4.1	6.5	191	10
Wh4	Downsweep	5.9	8.9	200	9
Wh5	Uphill	1.9	2.8	221	9
Wh6	Wail	2.2	3.9	203	3
Wh7	Loop	6.1	12.2	650	3

Given that there are a number of new whistle types identified from both humpback and snubfin dolphin acoustic recordings from the Kimberley and the existing acoustic repertoire for these species is from the east coast of Australia, there is clearly potential for geographic variation in the vocalization types for these species. However, no formal quantitative analysis has been undertaken as yet until the acoustic repertoire has been fully resolved and all available data has been analysed, which will occur when a more efficient detection algorithm for both species has been developed.

## 4 Discussion and Conclusions

### 4.1 Population genetic structure

The results presented here include an increased number of genetic samples and sampling sites over previous investigations of population genetic structure in snubfin and humpback dolphins in north-western Australian waters (Brown, Kopps, et al. 2014). Importantly, we were able to expand sample collection further east than

was previously achieved (Dampier Peninsula), into the more remote waters of the central and northern Kimberley.

With more samples from snubfin dolphins at both Roebuck and Cygnet Bay sites, we can corroborate Brown et al.'s (2014) findings of limited gene flow, with significant differentiation based on mtDNA and microsatellites and the symmetric proportion of contemporary migrants between populations estimated to be  $\leq 0.07$ . However, these updated results suggest slightly higher rates of contemporary migration than previously reported, with the upper 95% confidence intervals now at 0.10 and 0.12 – which is approximately at the 10% exchange threshold commonly cited for demographic dependence (Hastings 1993).

Results suggested greater levels of gene flow between snubfin dolphins from Cygnet Bay to Cone Bay than from Roebuck Bay to Cygnet Bay, with a lack of significant differentiation and the STRUCTURE analysis assigning the majority of individuals at Cone Bay and Cygnet Bay to the same genetic cluster. This higher level of gene flow can perhaps be explained by the two sites being separated by only c. 60 km of water across the top of King Sound, which may provide less of a barrier to dispersal than the c. 250 km of coastline between Cygnet and Roebuck Bays. Alternatively, these two populations may experience a stepping-stone pattern of gene flow through the distribution of animals throughout the c. 235 km coast of King Sound, which exhibits a continuous distribution of habitat in which snubfin dolphins are known to occur (shallow waters with muddy substrates, tidal creeks and extensive mangrove systems). By contrast, there appeared to be strong partitioning of snubfin dolphins sampled at Cone Bay and those at Yampi Sound, a location only c. 55 km along the coast to the north-east. Additional samples from Yampi Sound and other sites further east are required to further investigate this apparent differentiation and the factors that may contribute to genetic structure at a small geographic scale.

Results for humpback dolphins illustrated strong differentiation and very low gene flow between animals sampled at sites in the Pilbara and those at sites in the Kimberley, over 900 km to the north-east, with less pronounced structure between sites within regions. There was some evidence of differentiation of humpback dolphins between Cygnet Bay and Cone Bay, although this should be interpreted with caution due to the small number of samples for Cygnet Bay. The single humpback dolphin sample available for the Berkeley River allows very limited inferences to be made of population genetic structure between the western and eastern Kimberley; however, it is noted that even at higher values of  $K$ , this sample grouped with animals from Cone Bay, a location separated by some 800 km of highly complex coastline.

We were able to achieve our objective of gaining a better understanding of the genetic connectivity of snubfin and humpback dolphins in the Kimberley region to an extent. This included the collection of genetic samples from 12 humpback and 11 snubfin dolphins at the Cone Bay site, facilitating investigation of genetic differentiation between this site and those on the Dampier Peninsula and Pilbara coast. However, we had limited success in obtaining samples of either species from locations further east in the Kimberley. At these sites, two main factors contributed to fewer opportunities for sample collection: (1) shorter study durations than at other sites; and (2) animals being considerably less approachable. Additionally, sample collection at the Prince Regent River and Yampi Sound was secondary to other objectives and conducted with less experienced crews, further limiting opportunities for sampling. Possible reasons for the variability in the approachability of dolphins between study sites are discussed in Section 4.2 below.

## 4.2 Relative abundance

In this study, we collected data on encounter rates and the number of photo-identified snubfin and humpback dolphins at three sites (Cone Bay, Prince Regent River, Cambridge Gulf) where such data were not previously available. With the exception of Cone Bay, photo-ID data collected within this study were sparse; however, the comparison of encounter rates with published values for sites elsewhere in the Kimberley provides some indication of the relative abundance of dolphins in the sites surveyed here.

At all surveyed sites, encounter rates of snubfin dolphins were lower than those recorded at Cygnet Bay (0.23 dolphins/km) and Roebuck Bay (0.64 dolphins/km), which support local abundances of c. 50 and c. 140 snubfin dolphins, respectively (Brown et al. 2016). These comparisons suggest lower densities of snubfin dolphins at the surveyed sites than Cygnet or Roebuck Bay; however, differences in study area sizes and patterns of search effort may also influence these comparisons.

The moderate encounter rate of snubfins at Cone Bay (0.20 dolphins/km) and total of 27 photo-identified individuals suggests that this area is regularly used by a small number of snubfin dolphins. Furthermore, multiple re-sights of individual dolphins between 2014 and 2015 is consistent with the evidence of site fidelity

reported for other local populations (Parra, Corkeron, et al. 2006, Brown et al. 2016). An encounter rate of 0.19 snubfin dolphins/km within the Prince Regent River also suggests regular use by a small aggregation of animals, as is frequently reported by commercial vessels in the area (D. Barrow, Department of Parks and Wildlife, pers. comm., October 2016). Of the broader area, encompassing the adjacent Rothsay Water, Munster Water and Saint George Basin, the long, narrow inlet of the Prince Regent River and its adjacent creeks appear to most consistently be frequented by snubfin dolphins. With only four days of effort in the river, the eight distinctive individuals photo-identified is likely to be considerably lower than the number of animals which regularly use the area.

Encounter rates of snubfin dolphins within the Cambridge Gulf and adjacent coastal waters were among the lowest in the study. However, the broad area of survey effort and occasionally higher vessel speed (c. 14 knots) used while on survey effort is likely to have biased the encounter rate low in comparison to other, more enclosed sites, where a systematic route was followed. Surveying the northern section of the Cambridge Gulf and adjacent coastal waters yielded a considerably higher encounter rate of snubfin and humpback dolphins than the very sparse sightings (< 0.01 dolphins/km) that were recorded in the southern, inner sections of the Cambridge Gulf in 2012 (Brown et al. 2016). While snubfin dolphins were sighted throughout the area surveyed, most sightings were of single individuals and small groups, suggesting a widespread distribution and low density in the area. Insufficient photo-ID data were obtained for meaningful comparisons of the number of individuals with other sites.

Encounter rates of humpback dolphins were broadly similar to other surveyed sites in the western Kimberley, i.e. in the range of 0.05-0.15 dolphins/km. The highest encounter rate of 0.15 dolphins/km for the broader Prince Regent River area was strongly influenced by a single group of 10 humpback dolphins observed in Munster Water, although reports of regular sightings by Marine Park Rangers support the frequent occurrence of humpback dolphins in this area. The lowest encounter rate of 0.07 dolphins/km was recorded in Cone Bay, despite photo-identification surveys suggesting the regular use of this area by c. 15 distinctive individuals. As sightings maps reveal (Figure A1.2), humpback dolphins were frequently observed in larger groups in the vicinity of the fish farm in Cone Bay; this clustered distribution may have resulted in a lower probability of encountering dolphins while following an even pattern of survey effort.

The success of photo-ID varied between sites and species, and limited our ability to estimate the relative abundance of dolphins. At all sites, greater success was obtained for humpback dolphins than for snubfin dolphins, with the latter regularly showing strong vessel avoidance behaviour. The high photo-ID success rate (95%) for humpback dolphins encountered at Cone Bay in 2014 is comparable to that of Cygnet Bay (90%; Brown, unpublished data); we note that at both these sites dolphins are routinely exposed to small vessel movements associated with aquaculture operations and occasional recreational and tourism vessels. Similarly, photo-ID success was also greater for snubfin dolphins at Cone Bay (67%) than other sites, although falls some way short of the 94% and 86% recorded at Cygnet and Roebuck Bays, respectively (Brown, unpublished data). Both species were particularly difficult to approach in the Cambridge Gulf and adjacent coast, the location that receives relatively low levels of vessel traffic. The Prince Regent River, which experiences regular commercial tour vessels throughout the dry season (Apr-Oct), in addition to occasional recreational yachts and small numbers of commercial fishing vessels, was intermediate in terms of photo-ID success.

We hypothesize that across the sites sampled, in addition to others in the Kimberley region (Brown et al. 2016), routine exposure to vessel movements (which rarely deliberately approach dolphins) results in a level of dolphin habituation to small vessels, facilitating closer approaches and, therefore, greater success in obtaining photo-ID data and biopsy samples.

## 4.3 Passive Acoustic Monitoring

### 4.3.1 Soundscape

During the period when acoustic recordings were collected, the underwater acoustic environments within Roebuck Bay and Cygnet Bay were dominated by biological sources, namely snapping shrimp and fish choruses. However, vessel noise occurred intermittently at both locations and was often a result of the research vessel. When vessel noise was present in Roebuck Bay, it was often intense and considerably elevated background noise levels. During the middle of the dry season (July), vessel noise was present more often than at the end of the dry season in the lead-up to the 'build-up' season in September/October. The increased presence of vessel traffic is most likely due to an increase in tourism and boat-based activities during the cooler dry season in this

climate. The location of the acoustic receiver was within a channel with a sandbar on one side and a rocky, shallow bank (Black Ledge) on the other side. Hence, the channel is frequently used by boats in transit, particularly those traveling further east to Crab Creek which is a popular fishing spot. Black Ledge itself is also a popular fishing location for recreational and tourism-based fishing operations.

Roebuck Bay is also used as a safe mooring area for large vessels during the cyclone season and has several cyclone moorings that were within proximity of the acoustic receiver deployment site. The large tidal movement in this area means that the heavy-duty moorings moved within the water column as pressure was exerted on them by the high-velocity tidal stream. The tidal stream in Cygnet Bay also created significant noise, but mainly on the mooring that the acoustic receiver was attached to.

Most of the man-made noise within Roebuck Bay and Cygnet Bay was below the frequencies that dolphins produce sound (mostly above 1 kHz). Biological sources such as fish calls were also typically below 1 kHz. Thus limited overlap between anthropogenic and biological sound sources and dolphin sounds could be expected. The most prevalent sound source was snapping shrimp clicks. While these clicks do occur at frequencies overlapping with dolphin sounds, high intensity signals close to the receiver that could mask a dolphin sound are transient impulses. Consequently, these impulses would overlap very briefly.

#### 4.3.2 Vocal detections in association with dolphin occurrence

During the period of soundscape monitoring, three species of dolphins were observed during vessel surveys; snubfin, humpback, and bottlenose dolphins. In Roebuck Bay, snubfin dolphins were frequently observed, with this species present during all surveys. Bottlenose and humpback dolphins were rarely observed. The few sightings of bottlenose and humpback dolphins occurred south of the survey area and towards the middle of Roebuck Bay. Dolphin sightings within the small survey area (Stratum 2) with the acoustic logger, where most effort in July was placed, consisted of approximately 30% of all dolphin groups and individuals sighted. Only snubfin dolphins were sighted within this survey area, but were observed during all surveys. Dolphins were detected acoustically in approximately 67% of these surveys.

The survey stratum was designed to cover an area expected to be within acoustic detection range of dolphins within the site surveyed visually. Detection ranges for echolocation clicks estimated for other species and locations have been as high as approximately 1 km. With an acoustic receiver placed at the centre of the box-shaped survey area, the range along transects from the centre to the edge was 1.3 km. The radius from the centre was as large as 1.5 km to the corners of the survey area. There were a few dolphin sightings that were towards the edge of the survey, and could have been outside of the acoustic detection range.

Future work is recommended to: 1) model the acoustic detection range, and 2) compare the area determined to be within acoustic detection range (based on modeling) to visual observations within the area. To estimate the detection range of dolphins within the study area, propagation modelling, background noise levels (available from this study), characterization of sounds (also conducted in this study), source level measurements of sounds produced by dolphins at close range (that can be back-calculated to 1 m range), and the acoustic transmission environment would be required. The latter two parameters could be drawn from known information for similar species and environments where the data are lacking. However, these would be considered estimates with unmeasured associated uncertainty. To reduce uncertainty, measurements could be undertaken in future studies to obtain more accurate parameter estimates.

All surveys where only visual detections of dolphins but no acoustic detections occurred were during surveys with only one group sighted, all of which consisted of one individual with the exception of one group that had multiple animals. The lack of interaction with other animals when alone may be associated with a decreased production of sound. Thus, the probability of detection of an animal occurring alone during a short sample period would be reduced. In addition, the overall behaviour of animals affects the detection probability. Specifically, the duration in which animals remain in an area and the rate and level of sound production associated with activities directly affects the probability of detection.

During surveys, snubfin dolphins were observed socializing and foraging the majority of the time, often in large groups and remaining for extended periods within an area. Snubfin dolphin groups socializing or foraging are likely to produce sounds more frequently to communicate with each other and to locate prey. However, the frequency of sound production in groups with multiple animals likely highly variable among groups of different sizes as seen in this study. In contrast to snubfin dolphins, all observations of bottlenose and humpback dolphins observed during surveys in Roebuck Bay were in relatively small groups travelling fast in transit to

another location. Bottlenose and humpback dolphins remained for a short period within detection range. The limited acoustic detections of these species in hand-held hydrophone recordings from the vessel when dolphins were within visual detection range are most likely caused by their fast traveling behaviour and short duration within close proximity to the hydrophone. In summary, in using passive acoustics for monitoring dolphin density and occupancy, their behaviours, including the duration they spend in an area, group sizes in which they tend to occur in, and activities they undertake at different sites must be considered. All of these parameters affect the probability of detection.

Finally, in using passive acoustics for monitoring dolphins, there is always a trade-off in selecting the sample rate for recordings. A lower sample rate sufficient to capture most whistles under 20 kHz minimises file sizes of recordings, and allows longer or a greater number of recordings to be made. Alternatively, a higher sample rate allowing for frequencies above 20 kHz where most acoustic energy of echolocation and buzzes occurs can be selected. However, file sizes will be larger and fill up the hard drive more quickly, meaning that shorter or fewer recordings can be made. Because echolocation clicks and buzzes accounted for the majority of sounds detected, high sample rates to capture the high frequencies of these sounds are recommended for monitoring dolphins.

Overall, passive acoustic monitoring in Roebuck Bay in July was used successfully to document the presence of dolphins within the Stratum 2 survey area. A relatively short period of acoustic monitoring was required to detect dolphins. Vessel-based surveys within the area had a duration of approximately one to two hours. This period included 51 min to traverse the three transects within Stratum 2 plus the time taken off transect to collect photo-identification information. In recordings that detected dolphin sounds, the presence of dolphins during a vessel survey was confirmed in at least one ten-minute acoustic recordings (taken every 15 min by the acoustic logger), but usually dolphin sounds were present in multiple recordings. Thus, a two-hour sample period generally detected the occurrence of dolphins in groups with more than one individual successfully.

#### 4.3.3 Dolphin acoustic repertoire, behavioral context and geographic variation

In the literature, more has been published on humpback dolphin acoustics compared to snubfin dolphins although there is limited knowledge of the acoustic repertoire of these species, particularly with the relatively recent taxonomic reclassification of both species. The data presented here, while being a part of a larger study, has contributed in furthering our understanding of the acoustic repertoire of these species. There are two new humpback dolphin whistle types and one new snubfin dolphin whistle type that have so far been identified from the Kimberley acoustic data that are not represented within the literature. An essential component of PAM is to understand variation of dolphin vocalisations with respect to behaviour and geographical locations and obtain a comprehensive catalogue of their acoustic repertoire so that automatic detection algorithms can identify dolphin vocalisations within large acoustic datasets.

Using broadband hydrophones, we have also been able to identify ultrasonic harmonic components that extend in frequency range (up to 70kHz) beyond what has been identified in the literature for the whistles of humpback and snubfin dolphins. While this may not affect the dolphin vocalization detection process, it does provide for a more comprehensive understanding of the dolphins' acoustic repertoire and specifically determine whether either of the species produce ultrasonic whistles, which is present in other species. Generally, there was less variation found in the range of snubfin dolphin whistles compared to humpback dolphins and there also appears to be fewer different whistle types in the acoustic repertoire of snubfin dolphins. This potentially could mean that less variation in snubfin dolphin whistles may make it easier to train detection algorithms compared to humpback dolphins because there is less data to work with. The mean minimum frequency for humpback dolphins was 6.5kHz compared to 3.5kHz for snubfin dolphins, suggesting that snubfin dolphins potentially may produce whistles of lower frequency than humpback dolphins. This is not necessarily surprising though considering snubfin and humpback dolphins are often sympatric species and there may be a need to differentiate between the species in the same habitat, as has been found between humpback and bottlenose dolphin in Moreton Bay, Australia (Schultz & Corkeron 1994).

Given that there are a number of new whistle types identified from both humpback and snubfin dolphin acoustic recordings from the Kimberley and the existing acoustic repertoire for these species is from the east coast of Australia, there is clearly potential for geographic variation in the vocalization types for these species. However, no formal quantitative analysis has been undertaken as yet until the acoustic repertoire has been fully resolved and all available data has been analysed. Currently, a comprehensive comparison of humpback and snubfin dolphin data from different geographic areas is being undertaken on all available data.

Not surprisingly, there were low vocalization rates of humpback dolphins while travelling from one location to another within the survey area. Travelling behaviour does not require much communication between dolphins except to maintain group cohesion, predominantly when out of visual range. While humpback dolphins did not vocalise much during travelling, this was a prominent behaviour in all three of the Kimberley field sites. Consequently, the type of behaviour exhibited by dolphins has consequences on the choice of deployment location of the acoustic recorder and effectiveness of PAM. Humpback dolphins were found to be more vocal per unit of time whilst socializing and foraging compared to travelling and predominantly produced whistles while engaged in this behaviour. Socialising groups of dolphins will often have larger group sizes and the need for communication is greater due to more dynamic social interactions.

## 4.4 Conservation and management implications

### 4.4.1 Population genetic structure

We found slightly higher contemporary migration rates for snubfin dolphins between Cygnet Bay and Roebuck Bay than had previously been reported (Brown, Kopps, et al. 2014). However, levels of gene flow between the two sites are low, and the recommendation that animals in Roebuck Bay should be managed as a largely isolated and small local population remains appropriate. By contrast, the lack of significant genetic differentiation between snubfin dolphins at Cygnet Bay and Cone Bay suggests that the c. 50 snubfin dolphins apparently resident at Cygnet Bay could be considered part of a larger genetic population, encompassing animals from Cone Bay and, potentially, areas elsewhere in King Sound. The limited evidence of differentiation between these sites and animals sampled in Yampi Sound and further north/east suggests the existence of a third ancestral population, although additional sample collection in this region is required before such a conclusion may be drawn.

No humpback dolphin samples are available for over 900 km of coastline between Cygnet Bay and the Dampier Archipelago and, therefore, a more detailed picture of the structure and potential boundary between these two genetic populations is lacking. Nonetheless, these findings suggest at least two humpback dolphin management units in Western Australian waters, and will complement forthcoming abundance estimates for the Pilbara region (H. Raudino, Department of Parks and Wildlife, pers. comm., January 2017) in the development of appropriate conservation and management strategies.

For future genetic sample collection, consideration should be given to the challenges of obtaining samples from snubfin and humpback dolphins and the investment required in order to obtain meaningful sample sizes. Given the considerable costs and logistical difficulties of accessing most areas in the Kimberley region, opportunistic sample collection by collaborating with other operations such as Marine Park patrols and Ranger activities may present a cost-effective option. For example, further data collection by Marine Parks staff when operating in the Prince Regent, Talbot Bay and Yampi Sound areas. Similar opportunistic efforts may be considered during operations in the northern Kimberley Marine Parks once established. However, as our results have shown, dedicated trips with experienced crews and several weeks of sampling effort are typically required to obtain  $\geq 10$  samples of a species, even where animals are relatively approachable. Given the costs associated with longer, dedicated trips, it is recommended that any such future activities are focused in areas of particular management importance and/or those where there is considerable existing evidence of an abundance of animals which are relatively approachable.

Very little is known of the population genetic structure of snubfin and humpback dolphins across their wider distribution in northern Australian waters, with a notable gap in focused genetic sampling efforts between the east coast of Australia and those sites represented in the current study. Greater sampling effort is required to understand the appropriate geographic scales and sizes of populations across northern Australia in order to develop appropriate management measures at national, state/territory and regional levels.

### 4.4.2 Relative abundance

The identification of small, local populations of snubfin and humpback dolphins, with evidence of site fidelity, in Cone Bay provides useful data to inform environmental assessments of expanded aquaculture operations in the area (see Department of Fisheries 2013). Stratified surveys in Cone Bay showed humpback dolphins to occur throughout much of the study area (Figure A2.1); however, humpback dolphins were also frequently observed in close proximity to the aquaculture operations adjacent to Turtle Island. This study did not seek to examine associations between dolphin occurrence and aquaculture activities. Nonetheless, frequent

observations of the same humpback dolphin individuals foraging close to sea cages shows that fish farm aquaculture operations appear to be used as a foraging resource for humpback dolphins, comparable to their associations with fishing trawlers elsewhere in Australia (Parra 2006). New or expanded aquaculture operations in areas of known humpback dolphin occurrence should consider the potential interactions between the two, including increased vessel strike and entanglement risk. No such physical interactions were observed during the course of this study; however, dedicated empirical studies of the interactions between humpback dolphins and aquaculture operations would be required to properly investigate these potential risks.

Data on the relative abundance of inshore dolphins within the Prince Regent River area, while limited, confirmed the presence of snubfin, humpback and bottlenose dolphins within this narrow waterway. Given the relatively high frequency of vessel traffic and gillnet fishing activity in this area, management plans should establish and monitor the status of dolphins within the area. Furthermore, they should seek to determine the level of interactions between dolphins and gillnetting operations, which are a known cause of damaging levels of mortality to numerous small cetacean populations worldwide (Reeves et al. 2013). The collaborative nature of the survey in the Prince Regent River, involving researcher and key members of Lalang-garram/Camden Sound Marine Park Joint Management Team, facilitated progress towards an ongoing monitoring strategy. Training was provided in survey techniques and a recommended survey route was established. While the planned several days of survey effort once or twice a year will not permit statistically-robust detection of trends in abundance of dolphins, such effort will accumulate a better understanding of dolphins' use of this area. If continued over many years, as is planned, this monitoring may facilitate investigations of the long-term fidelity of specific individuals to the area, which is largely lacking for snubfin and humpback dolphins. Similarly, the participation of Dambimangari Rangers in data collection at Cone Bay (along with subsequent training and surveys coordinated by AMB in Yampi Sound) has provided both an interest and the capacity for ongoing surveys to add value to existing data for this site.

#### 4.4.3 PAM as a cost-effective tool for monitoring dolphin occurrence and habitat use

Roebuck Bay and Cygnet Bay are relatively pristine acoustic environments. The soundscape within these environments reflects the low human use of the sites. Thus, there is currently little overlap in dolphin sounds with man-made sounds that could limit the use of acoustics for detecting dolphins.

While the environment is ideal for PAM, because of the relatively high frequencies of dolphin sounds that attenuate quickly with range and moderate source levels, dolphins must be within proximity (tens to hundreds of meters) to be detected. Consequently, only with a grid or with mobile acoustic receivers (i.e. towed array) can the spatial scale over which PAM is effective for dolphins be comparable to other survey methods, such as boat or aerial-based surveys. Nevertheless, with the relatively cheap costs of acoustic receivers and continuous developments in acoustic survey technologies, there is improving capability to increase the spatial extent of acoustic survey areas. Therefore, PAM can be used as a cost-effective tool for monitoring the occurrence of humpback and snubfin dolphins. However, it is not possible yet to separate the species acoustically as the whistles and clicks for each species are still being quantified. As for many survey techniques, PAM has its biases and limitations, and prior information of how dolphins use the areas of interest to be surveyed would be important for the technology to be used to estimate relative abundance. Careful consideration about the research aims, environment and the target animals' behaviours should be made prior to the application of the technology for monitoring. In addition, further developments in processing large amounts of acoustic data through improving the accuracy of automatic detection routines are required for it to become a common survey tool.

PAM has its strengths in collecting data over long time periods (months or years) with minimal field work and associated costs required. In areas of high use by dolphins PAM can be applied effectively over short time periods (days, weeks and months) because recordings have sufficient detections to assess patterns in occurrence. In areas of low use by dolphins longer monitoring periods (many months) are required to acquire sufficient detections to assess patterns in occurrence. Moreover, it is desirable to have *a priori* knowledge of the dolphins' use of the area. Managers of marine areas are charged with the responsibility of implementing effective management of marine fauna. To do this, monitoring fauna over long periods is required and cost is always a limiting factor. To this point, PAM is typically less expensive than other survey methods (i.e. aerial or boat-based surveys), and is becoming more accessible with continuous improvements in acoustic technology and decreasing costs of acoustic receivers.

One of the current limitations to the use of PAM as a common survey method relates to the processing and analysis of the acoustic data. On the analysis side, it still needs to be resolved whether there is suitable and sufficient differentiation in the parameters of humpback and snubfin vocalisations to enable species identification. Recent work by Berg-Soto et al. (2014) demonstrates that various whistle parameters are capable of differentiating between snubfin and humpback dolphins. The data from this study, and the larger study led by JS, is expanding on this work to develop a comprehensive catalogue that will aid in the development of automatic detection algorithms for these species. Currently, another study in collaboration with JS is investigating the parameters of clicks for both snubfin and humpback dolphins to determine if there are any statistical differences between the species to be an effective basis for PAM. At this point in time, it is possible that there is not enough differentiation in the clicks between the two species to enable species identification. This has implications on the effectiveness of PAM because the types of vocalisations that are produced by dolphins will typically vary with behaviour. Generally, whistles and burst pulses are more often produced during socialising and milling behaviour whereas broadband clicks are produced during foraging behaviour. We found in the analysis of humpback dolphin vocalization rates that both whistles and clicks are prominent during socializing which provides the ability to conduct PAM through the identification of whistles to species. However, a limitation exists if socializing is not a significant component of their activity budget. Consequently, the choice of field site and the type of behaviour that can be observed there is an extremely important consideration in PAM.

Another current limitation that increases the cost of PAM in its application to snubfin and humpback dolphins is a lack of efficiency in automatic detection algorithms to identify dolphin vocalisations in acoustic datasets. Existing detection algorithms have been trained on more common species' of dolphins such as bottlenose, common and spinner dolphins. Current algorithms require a significant amount of manual checking to ensure appropriate quality control has been applied. Future work refining detection algorithms would significantly reduce current cost limitations in PAM.

## 5 References

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## 6 Communication

### 6.1 Students supported

WAMSI-supported data collection at Cone Bay in 2014 contributed to two chapters of the PhD thesis of Alexander Brown. Alex’s degree was conferred in March 2016, and his thesis is available to download from [Murdoch University’s Research Repository](#).

This WAMSI project also supported data collection in 2014 in Roebuck Bay used in one of the chapters of a PhD project carried out by Sarah Marley of Curtin University. Some of the soundscape analyses presented here were also used in her thesis to compare the soundscape of the relatively pristine environment in Roebuck Bay to the anthropogenic noise-rich environment in Fremantle, Western Australia. Sarah’s thesis was submitted in January 2017 and is currently under examination.

### 6.2 Journal publications

Data collected in Cone Bay in 2014 contributed to the following publication which addressed the WAMSI project objective of collecting data on the relative encounter rate, group size and composition of snubfin and humpback dolphins in the Kimberley Region:

- Brown, A.M., Bejder, L., Pollock, K.H. and Allen, S.J. (2016). Site-specific assessments of the abundance of three inshore dolphin species to inform conservation and management. *Frontiers in Marine Science* **3**: 4. doi: 10.3389/fmars.2016.00004

Photo-identification and sex data collected in Cone Bay in 2014 contributed to the following publications not directly associated with the WAMSI project objectives:

- Brown, A.M., Bejder, L., Parra, G.J., Cagnazzi, D., Hunt, T., Smith, J.L. and Allen, S.J. (2016). Sexual dimorphism and geographic variation in dorsal fin features of Australian humpback dolphins, *Sousa sahalensis*. *Advances in Marine Biology* **73**: 273-314. doi: 10.1016/bs.amb.2015.08.002
- Smith, F., Allen, S.J., Bejder, L. and Brown, A.M. (in press). Inferring predation risk from shark bite injuries on three species of tropical dolphins. *Marine Mammal Science* (accepted June 2017).

### 6.3 Proceedings/Technical Reports

Photo-identification and sex data collected in Cone Bay in 2014 contributed to the following conference poster presentations not directly associated with the WAMSI project objectives:

- Brown, A.M., Bejder, L., Parra, G.J., Cagnazzi, D., Hunt, T., Smith, J.L. and Allen, S.J. (2015). Sexual dimorphism in the dorsal fin features of Australian humpback dolphins: predicting sex from photo-ID images. *21<sup>st</sup> Biennial Conference of the Society for Marine Mammalogy, San Francisco, USA, 13-18 December 2015*.
- Smith, F., Allen, S.J., Bejder, L. and Brown, A.M. (2015). Inferring predation risk from the prevalence of shark bites among three tropical inshore dolphin species in north-western Australia. *21<sup>st</sup> Biennial Conference of the Society for Marine Mammalogy, San Francisco, USA, 13-18 December 2015*.

A summary of MUCRU and collaborators' research on snubfin and humpback dolphins in north-western Australia, including reference to WAMSI support, was included within the following conference presentation:

- Brown, A.M. and Bejder, L. (2016). Coastal dolphin research in Western Australia: Murdoch University Cetacean Research Unit's activities and key findings 2006-2016. *Australian Coastal Councils Conference 2016, 05 May 2016, Rockingham, Australia*.

### 6.4 Submitted Manuscripts

- Marley, S.A., Salgado Kent, C.P. and Erbe, C.A. (in review). Tale of Two Soundscapes: Comparing the acoustic characteristics of urban versus pristine coastal dolphin habitats in Western Australia. *Acoustics Australia*.

### 6.5 Presentations

The work and findings of the broader projects, to which WAMSI-supported data collection, have been communicated to a wide audience through several oral presentations to expert, public and stakeholder groups, including:

- CSK, March 2015: WAMSI North-Western Australian Marine Science Symposium, Fremantle, WA
- SJA, 2013: WAMSI North-Western Australian Marine Science Symposium, Fremantle, WA
- AMB, March 2014: Lunch and Learn presentation, Department of Parks and Wildlife, Kensington, WA
- AMB, April 2014: Public presentation towards Roebuck Bay Working Group Science on the Broome Coast Series, Broome Public Library, Broome, WA
- JS, April 2014: Presentation to the Bardi Jawi PBC describing the proposed research in Cygnet Bay in May 2014 and requesting permission to proceed.
- LB and CSK, March 2015: WAMSI conference, State Library of Western Australia, Perth, WA
- AMB, April 2015: Federally-funded workshop to progress national research framework of tropical inshore dolphin for their conservation and management, Department of the Environment, Canberra, ACT
- AMB, April 2015: Research overview presentation as part of Yawuru training workshop on dolphin surveys and data processing, Broome, WA
- AMB, June 2015: Presentation of WA inshore dolphin research towards James Cook University-led project with Dhimurru Aboriginal Corporation, Nhulunbuy, NT

- AMB, October 2016: Research overview presentation as part of Dambimangari Rangers training session, Dambimangari Aboriginal Corporation, Derby, WA
- AMB, August 2016: Research overview presentation as part of Balangarra Rangers training session, East Kimberley TAFE, Wyndham, WA
- SJA, October and November 2016: Two public presentations on “Dolphins of the Kimberley Coast” for The Kimberley – Like Nowhere Else, AH Bracks Library, Melville and Joondalup Library, Joondalup, WA

## 6.6 Other communications achievements

### 6.6.1 Articles in WAMSI Bulletins:

- [October 2014](#): reporting on Cone Bay 2014 photo-ID and biopsy data collection
- [October 2015](#): reporting on Cone Bay 2015 acoustic, photo-ID and biopsy data collection
- [October 2016](#): reporting on Cambridge Gulf photo-ID and biopsy data collection

### 6.6.2 Acknowledgement of WAMSI support in the following blog posts on the Murdoch University Cetacean Research Unit website:

- [PhD completion by Dr. Alex Brown: Inshore dolphins of northern Australia](#)
- [New publication on the abundance of inshore dolphins in north-western Australia](#)
- [New publication: Sexual dimorphism and geographic variation in dorsal fin features of Australian humpback dolphins](#)

### 6.6.3 Acknowledgement of WAMSI support in the following blog post on the University of Western Australia website:

- [New publication: Site-specific assessments of the abundance of three inshore dolphins](#)

### 6.6.4 Communications to Traditional Owners and local stakeholders

Several meetings with Traditional Owners (Bardi Jawi, Nyamba Buru Yawuru) and other local stakeholders (Roebuck Bay Working Group, DPaW, and DPaW Yawuru Rangers) occurred throughout the project. These meetings presented both a project overview to initiate working within Sea Country and updates, as well as opening discussions for future scientific work in the region.

A training session with Nyamba Buru Yawuru staff and rangers was held in August 2014. This training included a three-part interactive presentation which covered marine ecology, research methods, and working with dolphins. Training also included a ‘hands-on’ component, in which rangers were introduced to different pieces of research equipment (e.g. theodolite, acoustic noise loggers) to supplement their existing knowledge from previous fieldwork. The training session concluded with a discussion on the applicability of this knowledge, how to improve dolphin data collection, and future training opportunities. The training day was supplemented with a separate day of field work on the Yawuru/DPaW vessel to further develop their data collection techniques.

Social media was also used as a communication platform, with Yawuru posting updates on the August 2014 training session on their Facebook page. Radio Goolarri posted updates to their website, Facebook and Twitter pages promoting radio interviews project researchers discussing snubfin dolphins. Future information dissemination will include additional meetings with Traditional Owners and other stakeholders, further online presence, a media release, conference presentations and publications at later stages of analyses.

### 6.6.5 Associated media articles:

- [Kimberley dolphins vulnerable to human activity](#) (ABC Kimberley)
- [ABC TV News story by reporter Natalie Jones](#)
- [Rangers are getting to know our Kimberley dolphins](#) (Dambimangari Aboriginal Corporation)
- [Dolphin gender all in the fins](#) (ABC Radio interview with Vanessa Mills)
- [Dolphin count reveals homebody habits](#) (Science Network WA)
- [The Sound Environment](#) (Interview on Radio Goolari) July, 2014.

- [Snubfin dolphin behaviour and acoustics](#). (Interview on Radio Goolari) October, 2014.
- [Snubfin dolphin research](#) (Interview on ABC Kimberley Radio) October, 2014.

## 6.7 Knock-on opportunities created as a result of this project

The development of a positive relationship between Murdoch University researchers and Dambimangari Traditional Owners during fieldwork at Cone Bay contributed to further collaboration on the project: “Supporting Indigenous capacity to conduct inshore dolphin research and monitoring” (Chief investigator: AMB; Co-investigator: Dr. Isabel Beasley, James Cook University; Collaborators: WA Department of Parks and Wildlife). This project, funded by the Commonwealth Government and WWF-Australia, involved training sessions and a one-week dolphin survey in Yampi Sound with the Dambimangari Rangers in October 2016. Similar training activities took place with the Balangarra Rangers during the survey of the Cambridge Gulf in August 2016. Broader outputs from this project, which will be completed in 2017, include the development of a dolphin survey guidance document specific to indigenous ranger groups. Both Balangarra and Dambimangari Rangers are interested in further collaborative dolphin surveys in 2017.

Through the participation of DT on the Roebuck Bay Working Group and her links with NGOs and the Broome community developed over previous years working on snubfin dolphins in Roebuck Bay, new links between Curtin University researchers and Environs Kimberley, the Pew Charitable Trusts and Yawuru Traditional Owners have been created. In 2016, Broome-based NGO Environs Kimberley, with advice and support from DT, CSK and AB, facilitated the BBC’s Natural History Unit filming snubfin dolphin behaviour in Roebuck Bay and Cygnet Bay. The release of this media will bring greater national and international awareness of the conservation status of snubfin dolphins in Roebuck Bay and of the region.

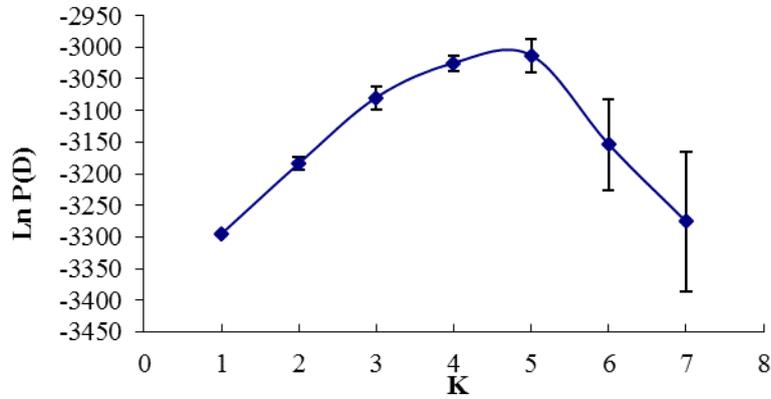
## 6.8 Key methods for uptake (i.e. advisory committee, working group, website compendium of best practice.)

Data collection activities with Dambimangari and Parks and Wildlife Rangers in the Prince Regent River resulted in recommendations being made for an ongoing dolphin monitoring program in the area. This will be facilitated through ongoing direct correspondence between AB, Rangers and DPaW Marine Park monitoring staff.

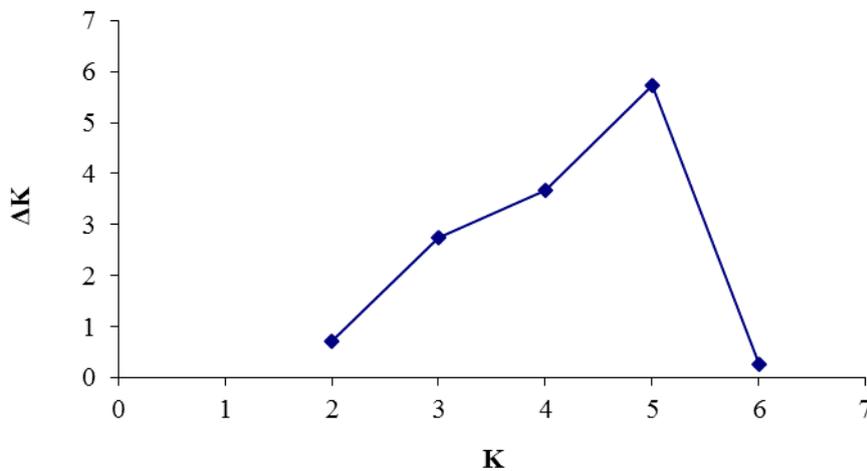
Jointly, Curtin and Murdoch Universities, DT and the WA Department of Parks and Wildlife have provided training and advice towards monthly dolphin surveys of Roebuck Bay led by Yawuru Traditional Owners from 2013-2015.

## 7 Appendices

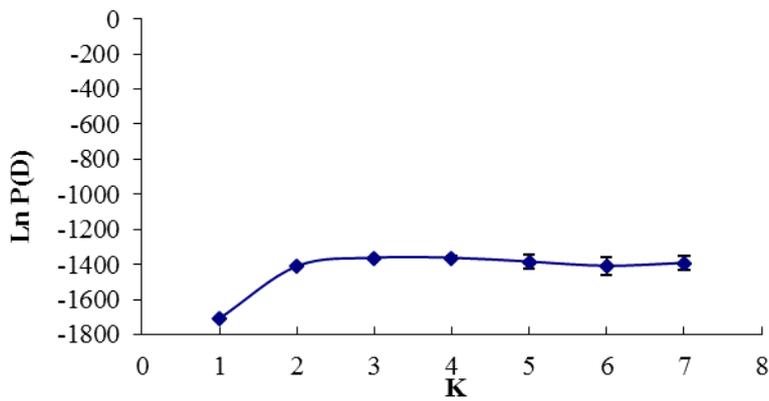
### Appendix 1. STRUCTURE results of population assignment



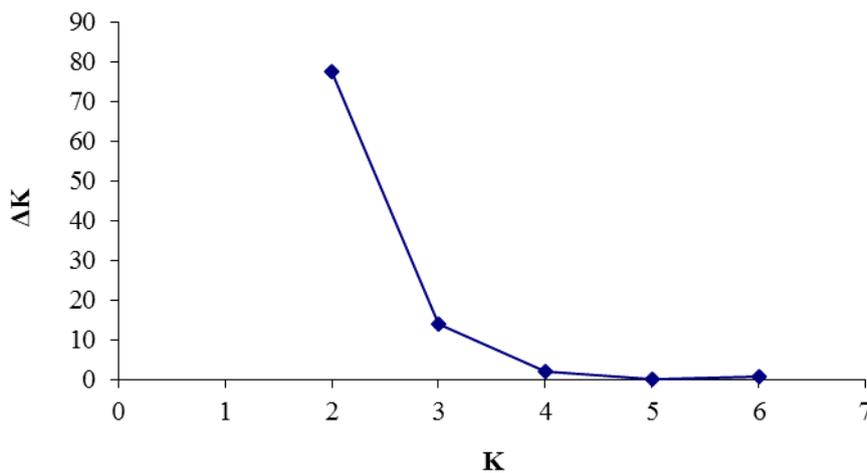
**Figure A1.1.** Snubfin dolphins. Plot of mean posterior probability (LnP(D)) values per cluster (K) generated by program STRUCTURE (Pritchard et al. 2000) based on 10 replicates per value of K with error bars indicating one standard deviation. Higher values of delta K indicate the optimum number of clusters (K). Data included microsatellite genotypes based on 11 loci for 106 snubfin dolphin individuals sampled at five putative populations.



**Figure A1.2.** Snubfin dolphins. Plot of delta K values per cluster (K) of mean posterior probability distributions according to Evanno et al. (2005). Higher values of delta K indicate the optimum number of clusters (K). Data included microsatellite genotypes based on 11 loci for 106 snubfin dolphin individuals sampled at five putative populations.

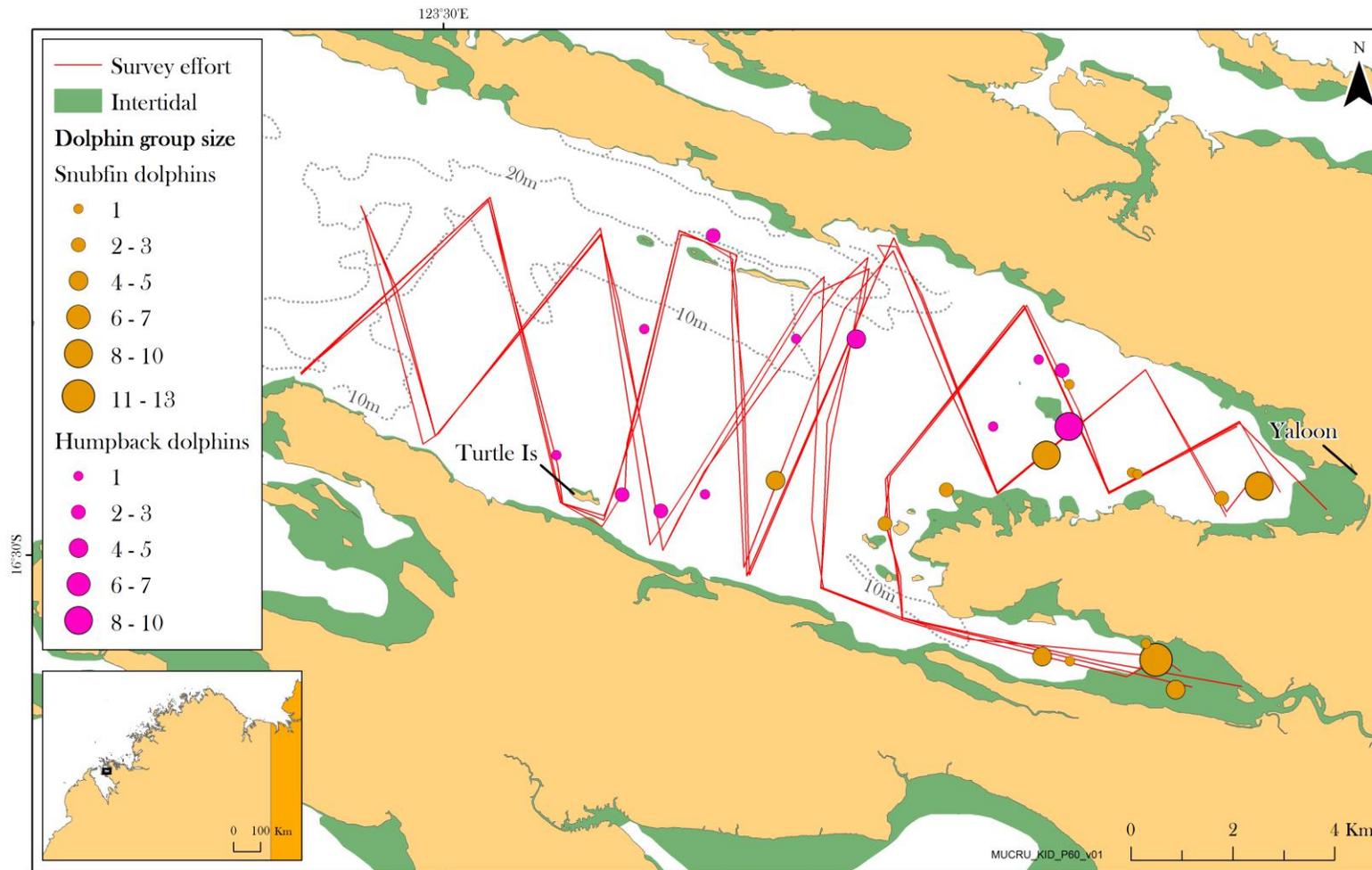


**Figure A1.3.** Humpback dolphins. Plot of mean posterior probability ( $\ln P(D)$ ) values per cluster ( $K$ ) generated by program STRUCTURE (Pritchard et al. 2000) based on 10 replicates per value of  $K$  with error bars indicating one standard deviation. Higher values of delta  $K$  indicate the optimum number of clusters ( $K$ ). Data included microsatellite genotypes based on 13 loci for 54 humpback dolphin individuals sampled at five putative populations.

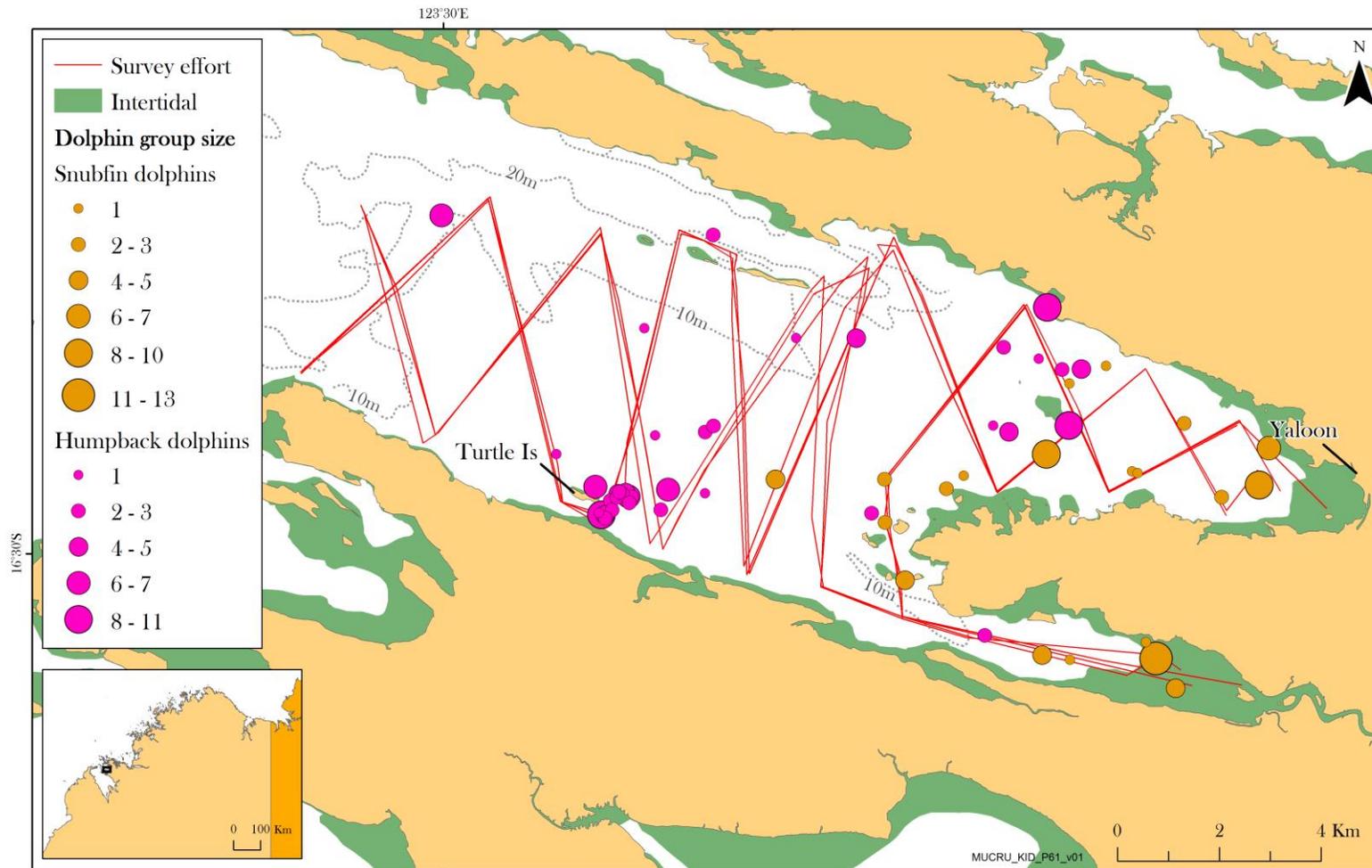


**Figure A1.3.** Humpback dolphins. Plot of delta  $K$  values per cluster ( $K$ ) of mean posterior probability distributions according to Evanno et al. (2005). Higher values of delta  $K$  indicate the optimum number of clusters ( $K$ ). Data included microsatellite genotypes based on 13 loci for 54 humpback dolphin individuals sampled at five putative populations.

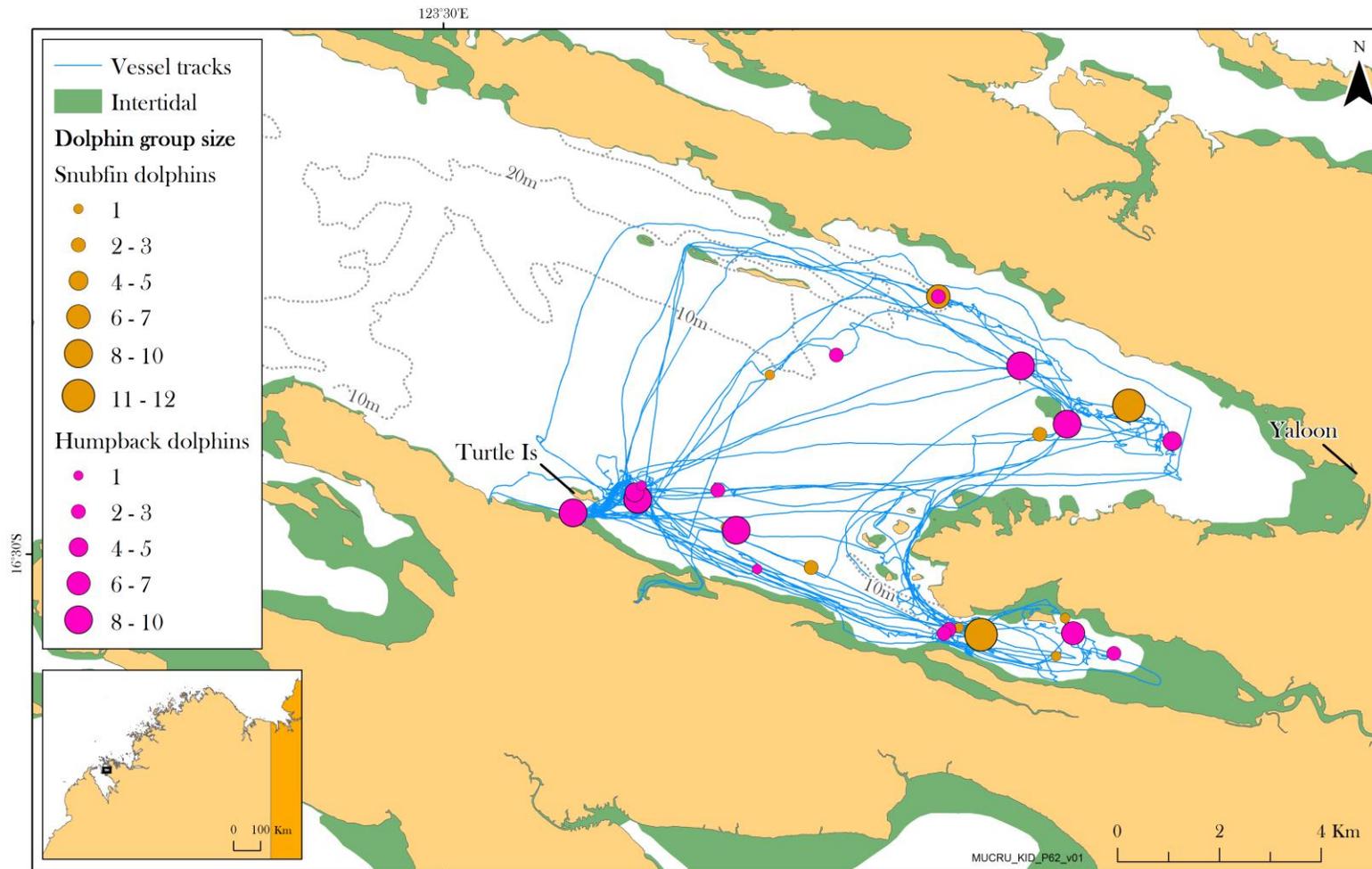
## Appendix 2. Dolphin sighting maps for all sites



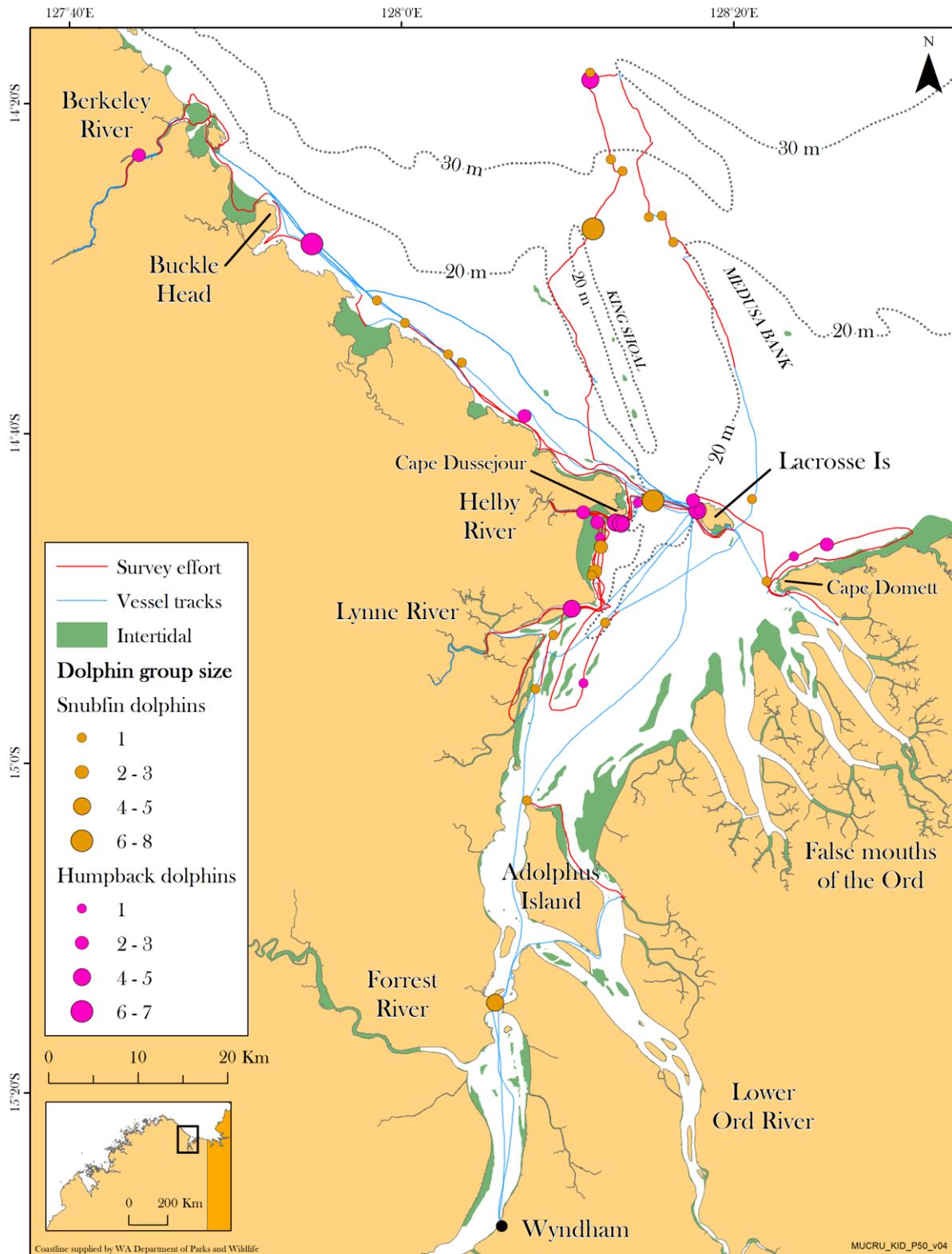
**Figure A2.1.** Sighting locations and group sizes of snubfin and humpback dolphins in Cone Bay, 2014, including transect survey effort. Only dolphins observed while on transect survey effort are shown; these represent those used to calculate encounter rates. Fish farm infrastructure (sea cages, moorings and vessels) extends c. 1 km east of Turtle Island.



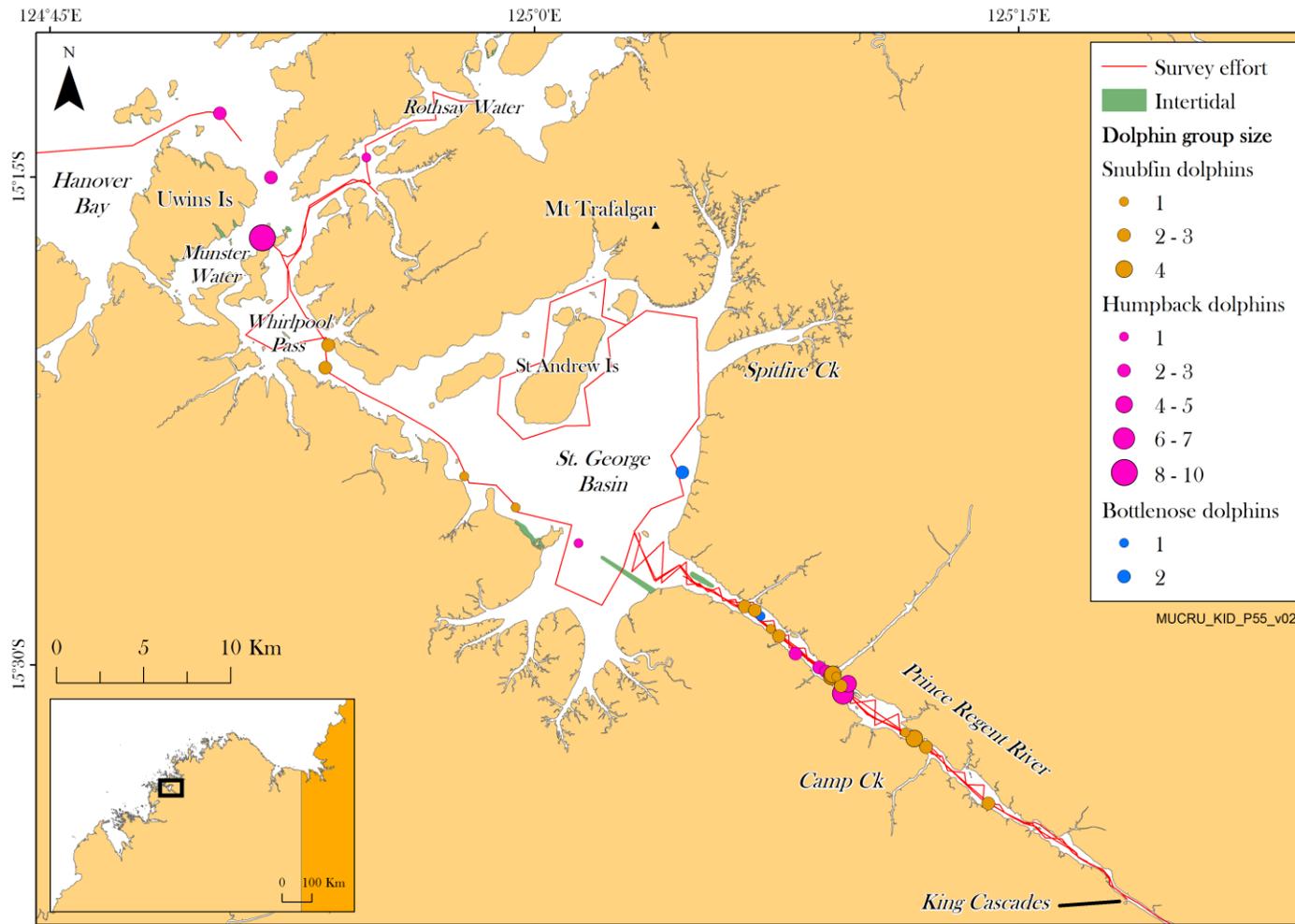
**Figure A2.2.** Sighting locations and group sizes of snubfin and humpback dolphins in Cone Bay, 2014, including transect survey effort. All dolphin sightings are shown, including those observed on transect effort and opportunistically. Fish farm infrastructure (sea cages, moorings and vessels) extends c. 1 km east of Turtle Island. Considerable time was spent attempting to biopsy sample dolphins around the fish farm infrastructure when not completing transect survey effort.



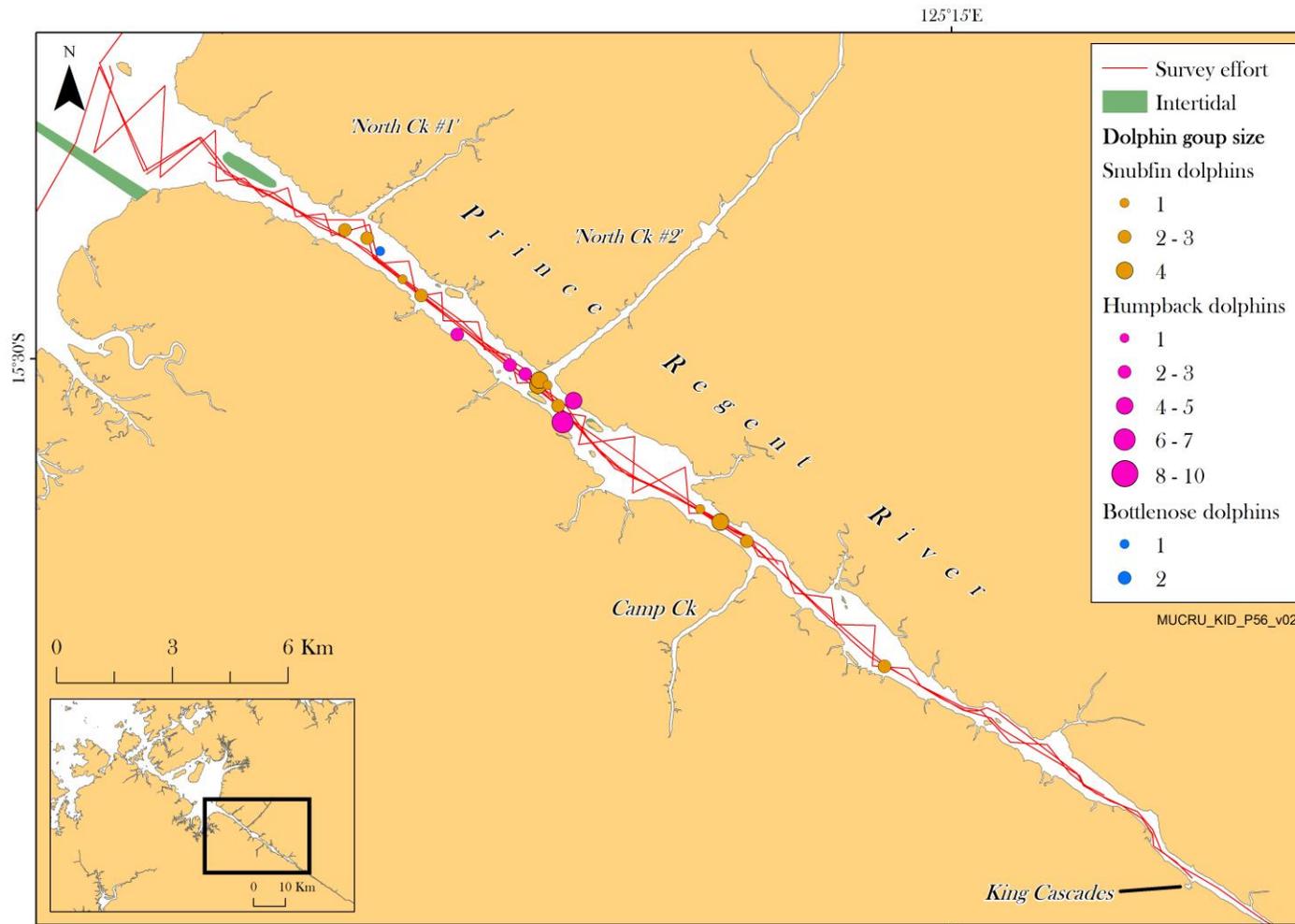
**Figure A2.3.** Boat-based sighting locations and group sizes of snubfin and humpback dolphins in Cone Bay, 2015, including vessel tracks. No survey effort was recorded due to the limited number of observers present (two) and primary objective of collecting acoustic data. Fish farm infrastructure (sea cages, moorings and vessels) extends c. 1 km east of Turtle Island.



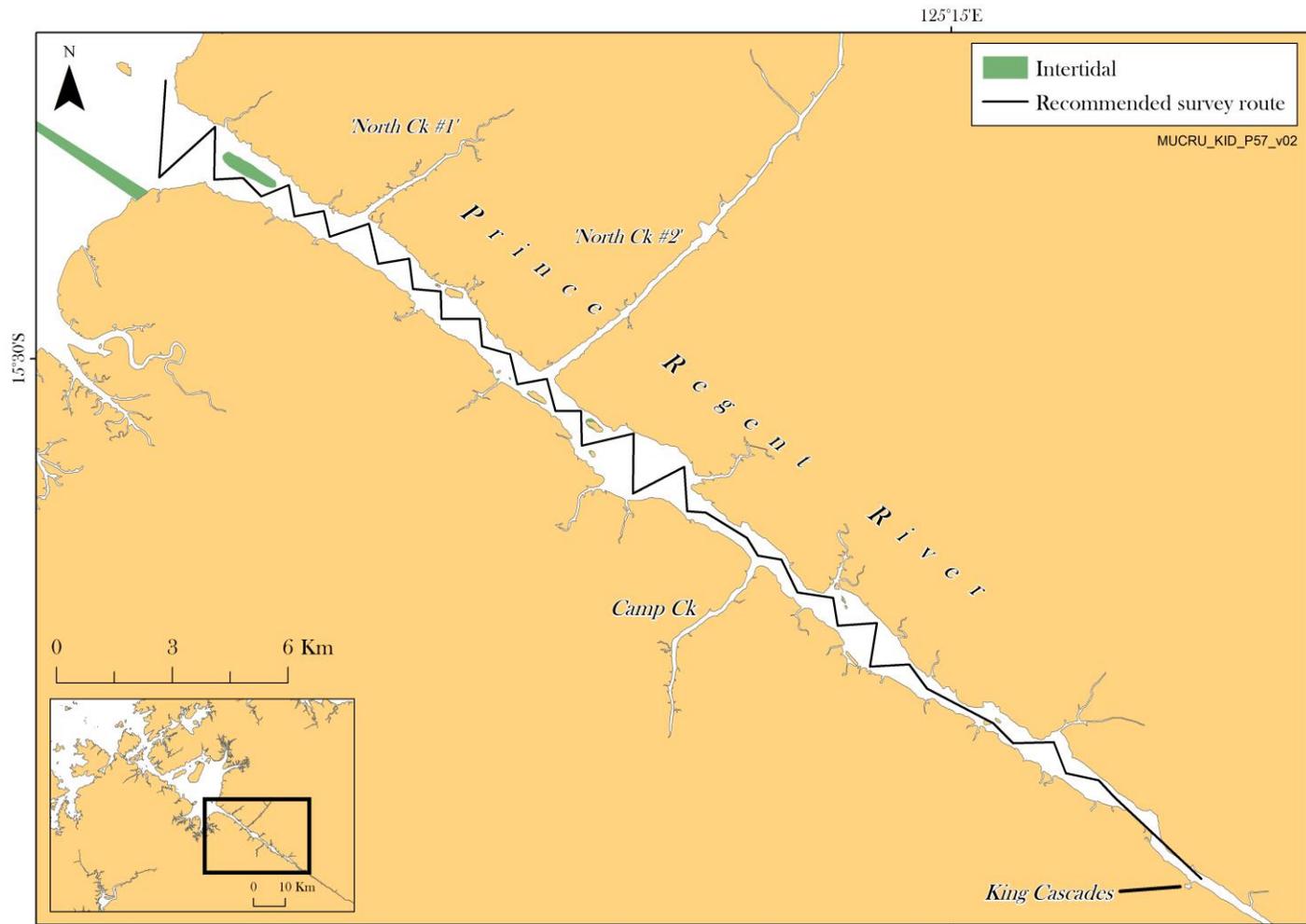
**Figure A2.4.** Sighting locations and group sizes of snubfin and humpback dolphins in the Cambridge Gulf, August 2016, including vessel tracks and survey effort. Dolphin sightings observed on survey effort and opportunistically are shown. Two of the three sightings on the west coast of Lacrosse Island were observed from land.



**Figure A2.5.** Sighting locations and group sizes of snubfin, humpback and bottlenose dolphins in the Rothsay Water to Prince Regent River area, September 2016, including survey effort. Dolphins observed on transect effort and opportunistically are shown. Survey effort extended a further 10 km upstream from King Cascades, with no dolphin sightings; this effort is not considered within the calculation of encounter rates, as the shallow, narrow channel is difficult to navigate and is unlikely to regularly support dolphins. For the calculation of encounter rates for the Rothsay Water to Prince Regent River area, survey effort west of Uwins Is. is not included.

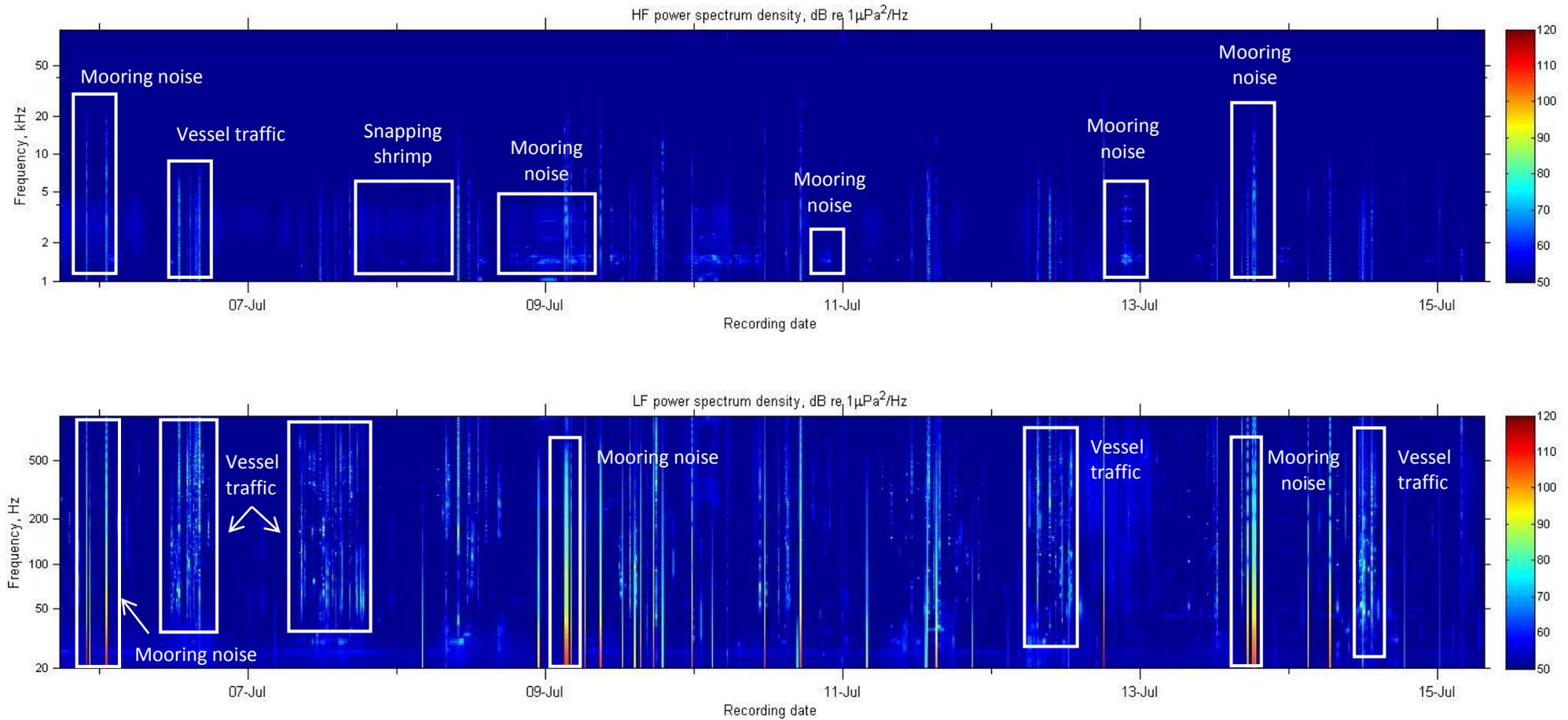


**Figure A2.6.** Sighting locations and group sizes of snubfin, humpback and bottlenose dolphins in the Prince Regent River, September 2016, including survey effort. Dolphins observed on transect effort and opportunistically are shown. Survey effort extended a further 10 km upstream from King Cascades, with no dolphin sightings; this effort is not considered within the calculation of encounter rates, as the shallow, narrow channel is difficult to navigate and is unlikely to regularly support dolphins.

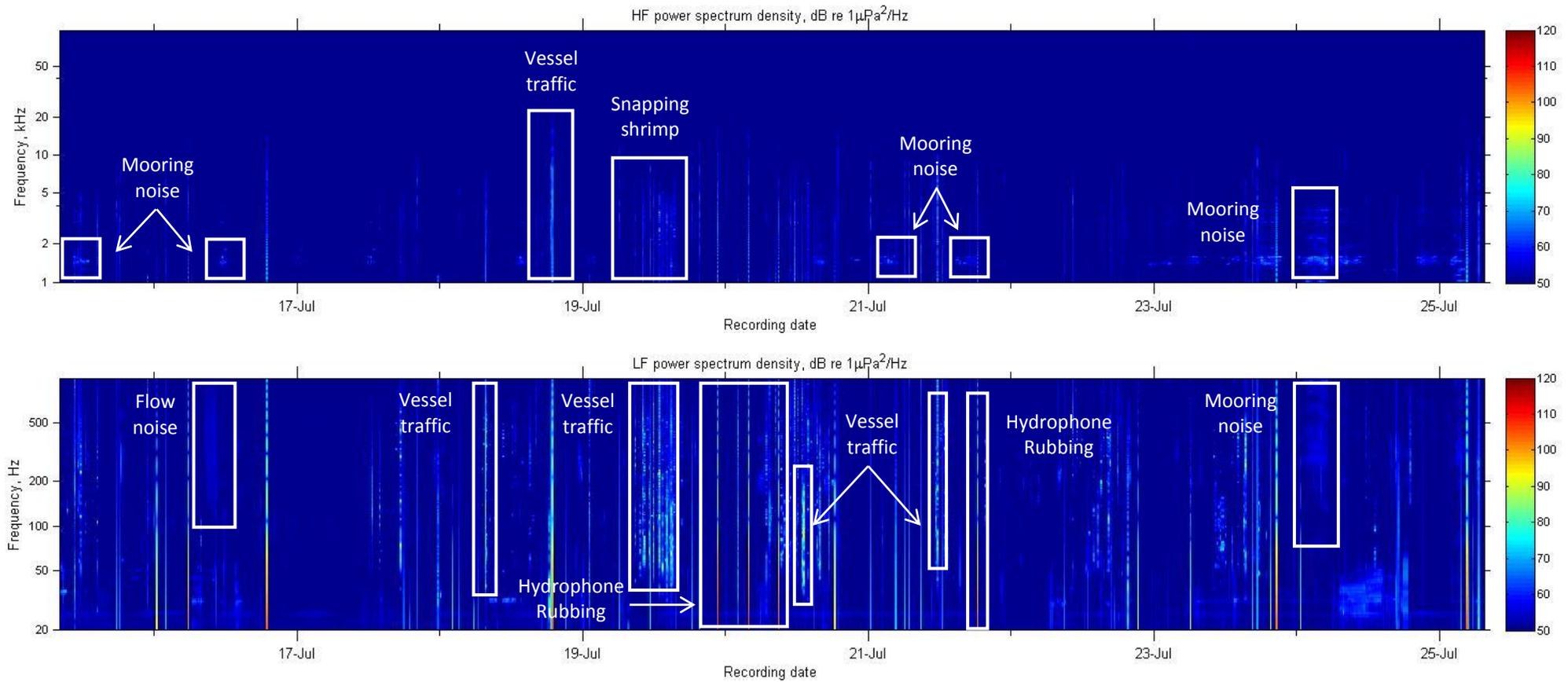


**Figure A2.7.** The recommended survey route for future dolphin monitoring surveys in the Prince Regent River, to be led by Parks and Wildlife and Dambimangari Joint Management Rangers. This route, of 46.8 km length from the mouth of the PRR to King Cascades, cover some 32 km of the waterway. This length of route should be completable within a single good weather day given moderate numbers of dolphin sightings, and can therefore be repeated at least three times within a research cruise to the area.

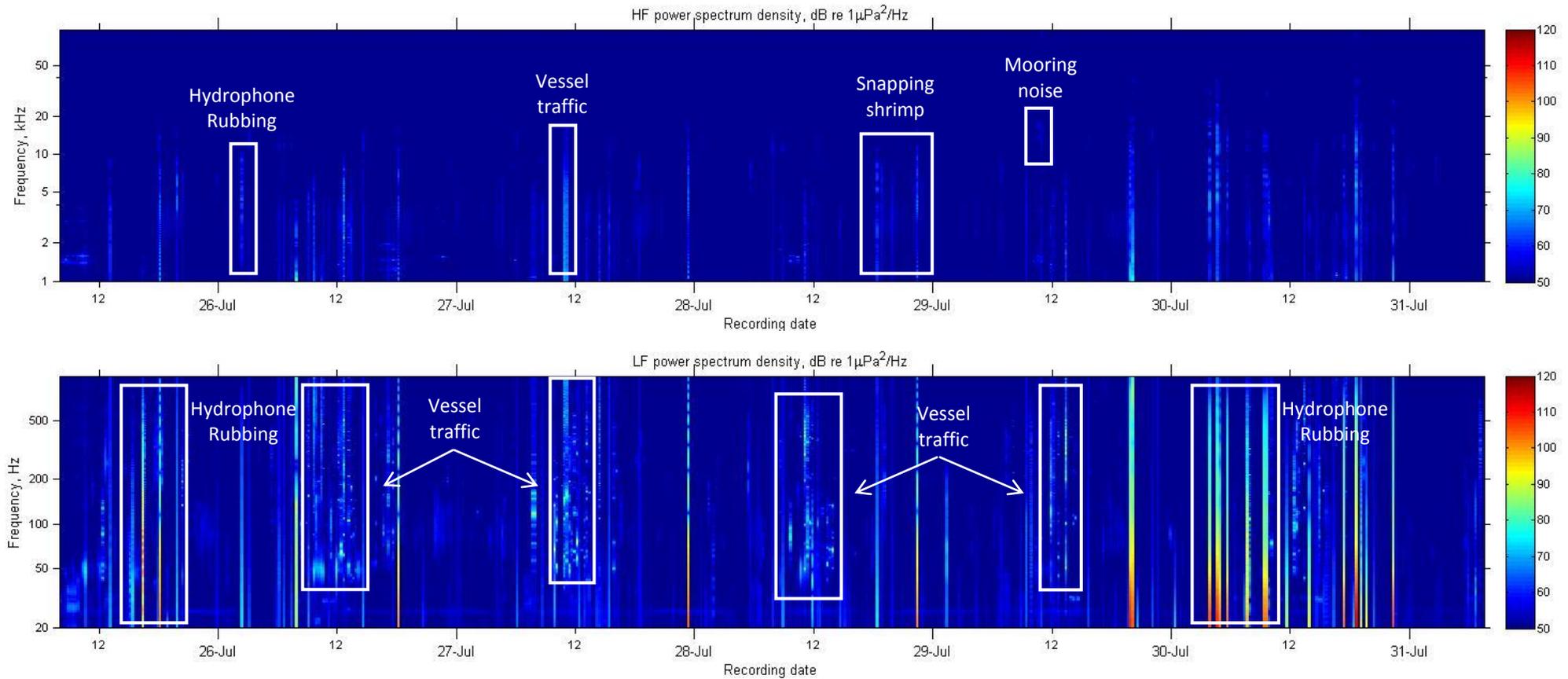
### Appendix 3. Roebuck Bay soundscape spectrograms



**Figure A3.1.** Spectrograms of the first 10-day period of recordings made in Roebuck Bay during July 2014, showing the high (top) and low (bottom) frequency ranges.

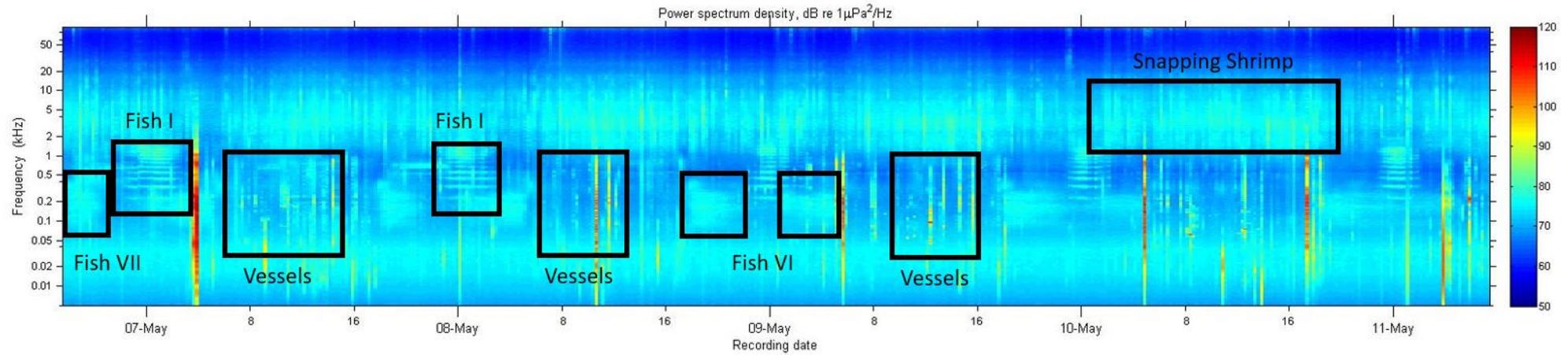


**Figure A3.2.** Spectrograms of the second 10-day period of recordings made in Roebuck Bay during July 2014, showing the high (top) and low (bottom) frequency ranges.

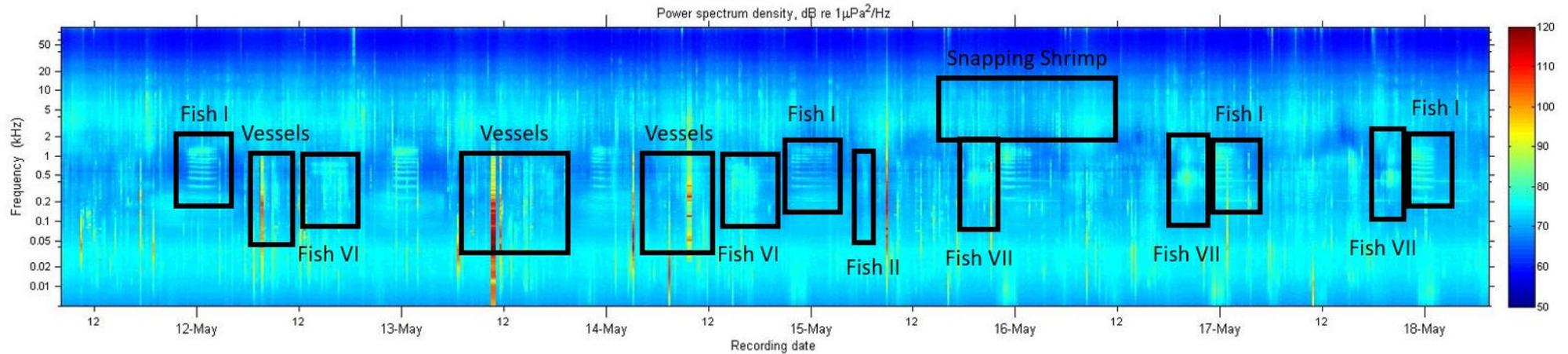


**Figure A3.3.** Spectrograms of the final 6-day period of recordings made in Roebuck Bay during July 2014, showing the high (top) and low (bottom) frequency ranges.

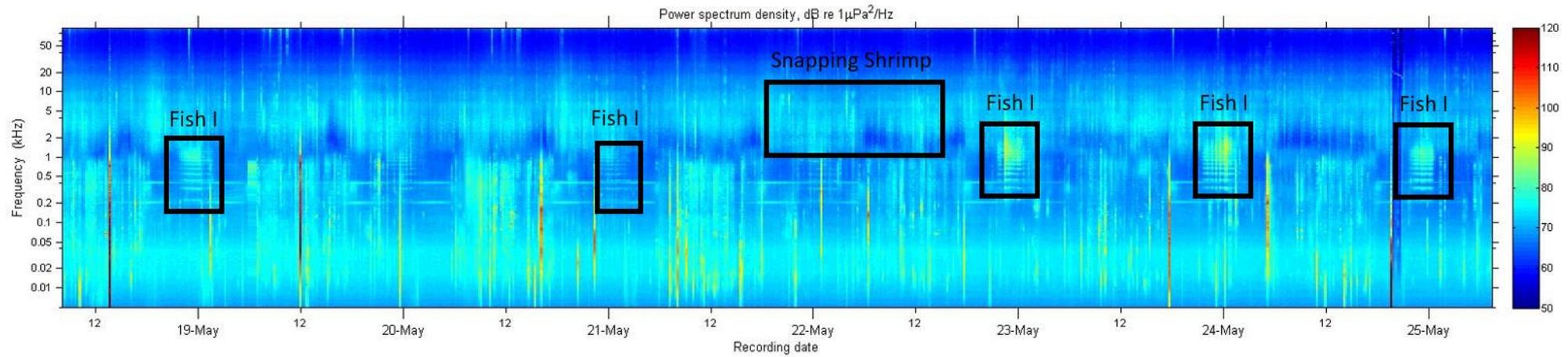
#### Appendix 4. Cygnet Bay soundscape spectrograms



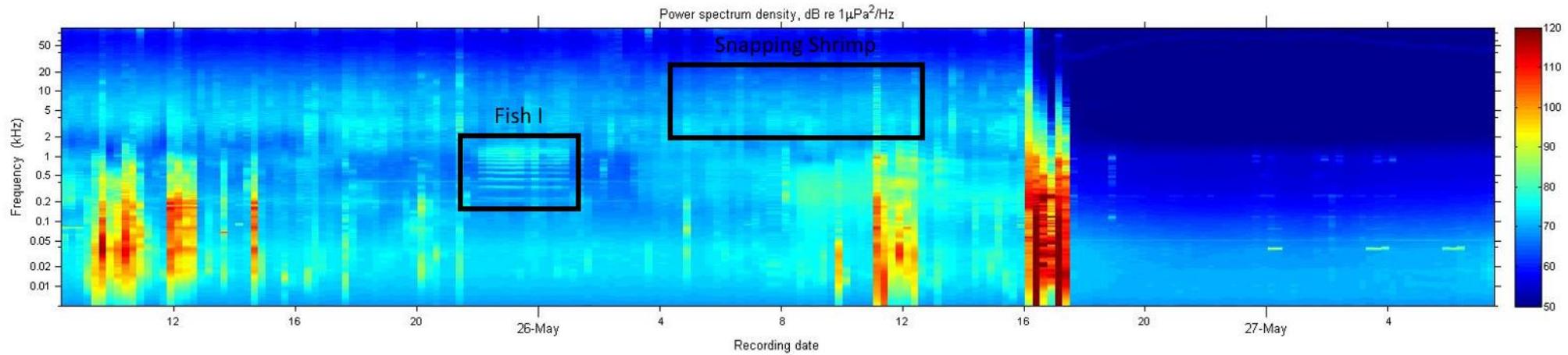
**Figure A4.1.** Spectrograms of the first 5-day period of recordings made in Cygnet Bay during May 2014.



**Figure A4.2.** Spectrograms of the following 7-day period of recordings made in Cygnet Bay during May 2014.



**Figure A4.3.** Spectrograms of the third 7-day period of recordings made in Cygnet Bay during May 2014.



**Figure A4.4.** Spectrograms of the last 3-day period of recordings made in Cygnet Bay during May 2014.

Appendix 5. Dolphin sightings in Roebuck Bay as part of the Acoustic study

Figure A5.1. Dolphin sightings in Roebuck Bay by species and group size

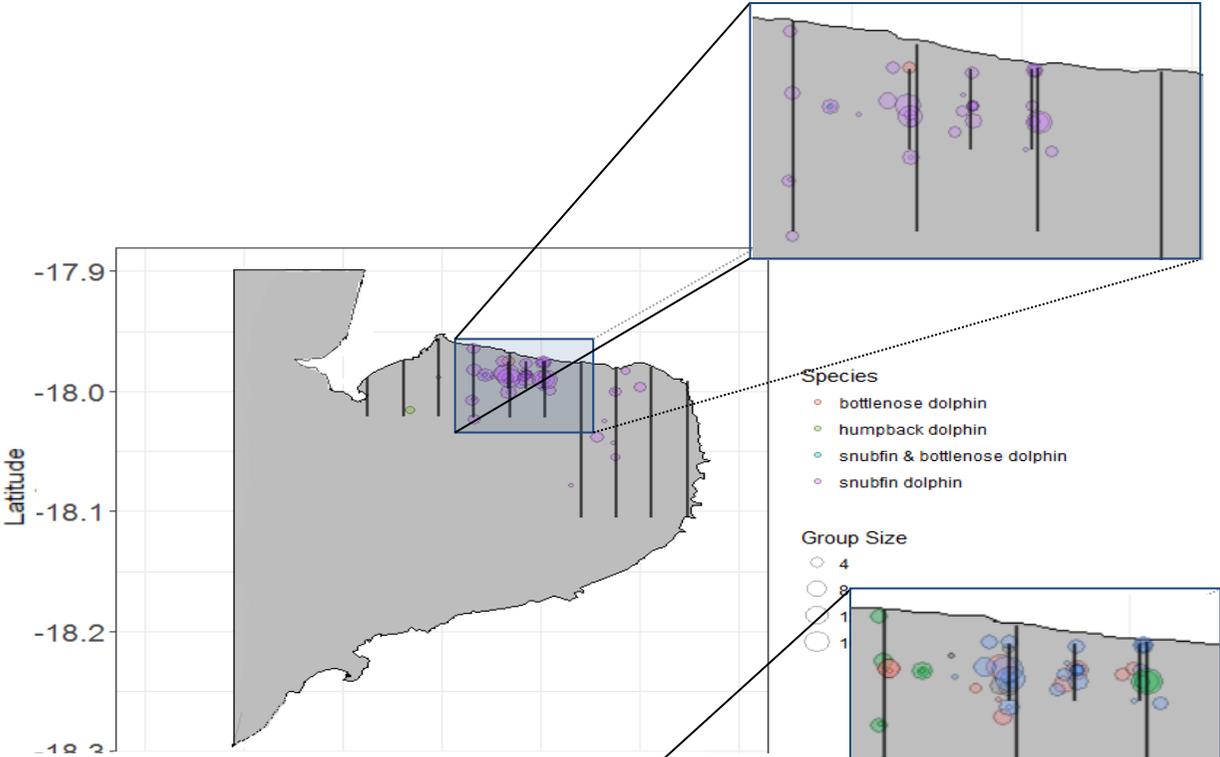
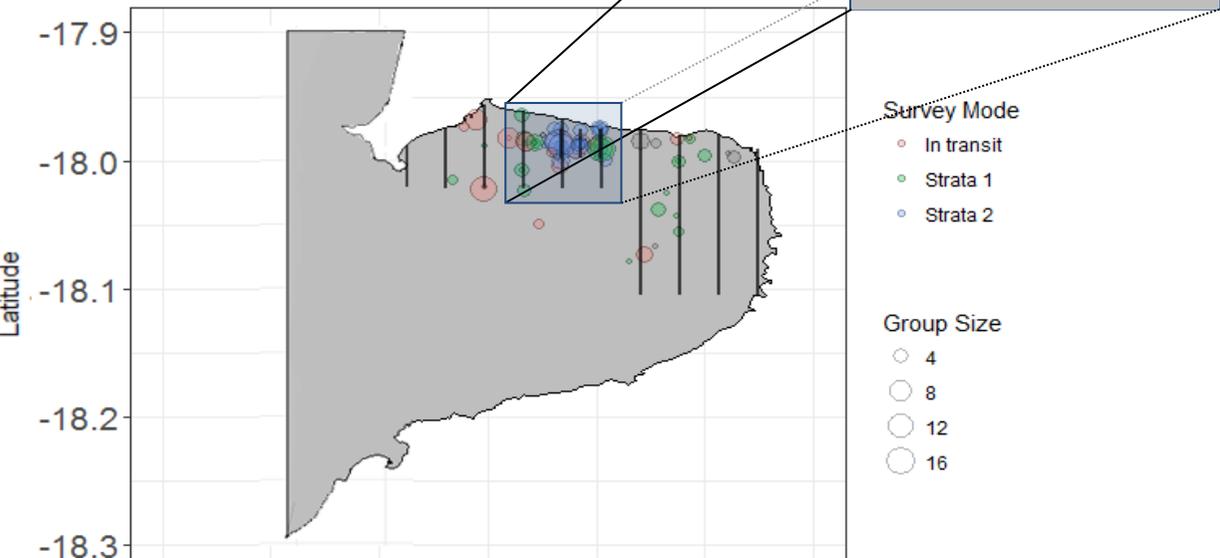


Figure A5.2. Dolphin sightings (all species) in Roebuck Bay by Sample M



## Appendix 6. Responses to questions outlined in the Kimberley Marine Research Program Science Plan.

### Key Question

Informed Response

#### **What are the priority species of coastal dolphins in the Kimberley and why?**

Australian snubfin (*Orcaella heinsohni*) and Australian humpback (*Sousa sahulensis*) dolphins are priority species due to their limited global distribution, apparent low numbers, fragmented population structure, and reliance upon near-shore habitats. While globally widespread and more abundant, Indo-Pacific bottlenose dolphins also occur in coastal waters of the Kimberley, including many near-shore habitats, and are vulnerable to the same threatening processes.

#### **What are the distribution, abundance and movement patterns of these populations?**

Snubfin and humpback dolphins are broadly distributed throughout coastal waters of the Kimberley, and were observed at all surveyed sites. Finer-scale habitat selection has yet to be investigate. In this study, both species were observed in a variety of shallow-water ( $\leq 30\text{m}$ ) habitats, including: tidal inlets and creeks; sheltered bays with mangroves; exposed stretches of open rocky coast; and shallow sand and mud habitats extending up to 35 km offshore.

Our results only indicate the relative abundance of snubfin and humpback dolphins in selected areas. Determining absolute abundance requires far greater survey effort and was not within the scope of this study. Nonetheless, our data suggest local abundances (i.e. numbers of dolphins within a defined bay, inlet or estuary) to be comparable to or less than most sites elsewhere in the Kimberley for which absolute abundance has been estimated (i.e.  $\leq c. 100$  individuals, typically  $\leq 50$ ). The total abundance for either species across the Kimberley as a whole remains unknown.

Our genetic results can, to some extent, infer the historical movement of animals between sampled sites. For both snubfin and humpback dolphins, mitochondrial and nuclear genetic markers suggested limited movement between some sites separated by several hundred kilometres, but that patterns of dispersal are likely complex and influenced by the nature of the habitat between sampled populations.

Photo-identification data revealed a humpback dolphin female and calf to move along at least 52km of coastline within three days. However, matches of individuals between any two surveyed sites in the Kimberley (the closest of which are c. 60km distant) have not been observed. Indeed, photo-identification data from Cone Bay provide evidence of site fidelity for both species, similar to findings from sites elsewhere in the Kimberley.

#### **What, when and where are their critical habitats?**

This study did not conduct the regional-scale stratified survey effort required to reliably identify areas of greater and lesser importance to snubfin and humpback dolphins. Nonetheless, the aggregations of snubfin dolphins in Roebuck Bay and Cygnet Bay appear to be somewhat exceptional, and further investigation of the habitat characteristics of these locations may provide indications of what constitutes critical habitat for snubfin dolphin.

The temporal extent of available data is limited, and temporal variation in habitat use has not yet been comprehensively studied for snubfin or humpback dolphins. However, evidence of strong site fidelity and observations of foraging and sexually-oriented socialising behaviour at different times of the year in the same areas suggest that there is limited seasonality in the nature of critical habitats.

#### **Why are these areas important?**

These areas are important because they provide habitat for activities which are essential to the survival and reproduction of snubfin and humpback dolphins: feeding, resting, breeding and rearing young.

**What are the appropriate spatial management units for priority species?**

Further data are required to comprehensively characterise spatial management units for snubfin and humpback dolphins in the Kimberley region. Our results identified two snubfin dolphins genetic clusters: (1) Roebuck Bay; and (2) Cygnet Bay-Cone Bay; based on the limited gene flow between these two clusters and a precautionary approach, it is recommended that they are treated as separate management units, albeit with a low but important level of genetic connectivity. We also provide limited evidence for a possible third genetic cluster to the north-east of Cone Bay, based on two samples from Yampi Sound and one from the Prince Regent River. Genetic results for humpback dolphins in the Kimberley are limited, although there is evidence of strong differentiation between sampled sites in the Pilbara and Kimberley regions, such that they should be regarded as separate management units.

**What environmental factors are ‘driving’ the above distribution patterns and population characteristics?**

This remains unknown, and was not within the scope of the study. As with most small cetaceans, the distribution of snubfin and humpback dolphins will be largely driven by the availability of food resources, predation risk and breeding opportunities. Modelling correlations between spatial distribution of dolphins and environmental conditions could provide insight into factors influencing their distribution. Future work is planned to use existing data from Roebuck Bay for habitat modelling to identify potential drivers of snubfin dolphin distribution.

**How will they likely respond to climate change?**

The data collected in this study do not support any inferences on how snubfin and humpback dolphins in the Kimberley may respond to climate change.

**What are the major pressures on marine fauna in this region and how can they be measured using key indicators over the long-term (e.g. marine debris)**

Threats to, and actual impacts on, snubfin and humpback dolphins in this region are poorly characterised, as is the case across much of northern Australia. However, known threats to small cetaceans in coastal environments include: habitat modification and loss; entanglement in marine debris and fishing gear (particularly gillnets); prey depletion from fisheries; poor water quality (e.g. high concentrations of anthropogenic contaminants); vessel collisions; and, physical and acoustic disturbance.

Throughout much of the Kimberley region, most of the aforementioned threats are likely to be low. However, there will be a greater risk of impacts in local areas experiencing higher levels of human activity such as commercial fishing, vessel traffic and coastal development.

**What role can marine fauna play in identifying areas of high productivity (e.g. tracking key species to hotspots).**

Given the high daily prey intake requirements for small cetaceans, any aggregations of these species could be considered areas of high productivity.

**What cost-effective methods can be developed to enable effective condition monitoring of priority species.**

PAM has its strengths in collecting data over long time periods (months or years) with minimal field work and associated costs required. Currently, PAM can effectively be used to monitor occurrence of vocally active animals over relatively long periods of time. With a grid of receivers, the distribution and movement of vocally active animals can be described, and used to model habitat use and identify environmental drivers. To extend the range of applications that PAM can be used in (for instance species specific abundance estimations), further developments are required. For instance, improvements in the accuracy of automatic detection algorithms, more work to identify species based on acoustics accurately, and development of methods for estimating absolute abundance is required.

Obtaining robust data on the abundance or even relative abundance of these rare dolphin species in an area over time is difficult and requires considerable resources. Intensive boat-based capture-recapture studies repeated across multiple years have proven somewhat effective in monitoring inshore dolphins in the Darwin Harbour Region (see Brooks et al. 2017), although they appear ineffective in areas of low dolphin abundance.

To be cost-effective in WA, such studies would need to focus on more accessible locations with a known abundance of the focal species. For example, at Roebuck Bay, intensive boat-based sampling (i.e. multiple vessels operating simultaneously over several weeks), repeated at regular intervals over a number of years, should provide robust information on the abundance of snubfin dolphins over time. Where only a lower intensity of effort is possible and animals appear less abundant, such as in the Prince Regent River, repeated stratified boat-based sighting surveys could provide useful information on major changes in relative abundance over time. This could perhaps be sufficient to alert further investigation using more intensive sampling should concerns on the condition of animals in the area be raised.

**On what scales are large marine fauna connected both within and outside the Kimberley (genetics, tracking, tagging)?**

See comments on movements and spatial management scales (above).