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Genetic isolation between coastal and fishery-impacted, offshore bottlenose dolphin (*Tursiops* spp.) populations

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Abstract

The identification of species and population boundaries is important in both evolutionary and conservation biology. In recent years, new population genetic and computational methods for estimating population parameters and testing hypotheses in a quantitative manner have emerged. Using a Bayesian framework and a quantitative model-testing approach, we evaluated the species status and genetic connectedness of bottlenose dolphin (*Tursiops* spp.) populations off remote northwestern Australia, with a focus on pelagic 'offshore' dolphins subject to incidental capture in a trawl fishery. We analysed 71 dolphin samples from three sites beyond the 50 m depth contour (the inshore boundary of the fishery) and up to 170 km offshore, including incidentally caught and free-ranging individuals associating with trawl vessels, and 273 dolphins sampled at 12 coastal sites inshore of the 50 m depth contour and within 10 km of the coast. Results from 19 nuclear microsatellite markers showed significant population structure between dolphins from within the fishery and coastal sites, but also among dolphins from coastal sites, identifying three coastal populations. Moreover, we found no current or historic gene flow into the offshore population in the region of the fishery, indicating a complete lack of recruitment from coastal sites. Mitochondrial DNA corroborated our findings of genetic isolation between dolphins from the offshore population and coastal sites. Most offshore individuals formed a monophyletic clade with common bottlenose dolphins (*T. truncatus*), while all 273 individuals sampled coastally formed a well-supported clade of Indo-Pacific bottlenose dolphins (*T. aduncus*). By including a quantitative modelling approach, our study explicitly took evolutionary processes into account for informing the conservation and management of protected species. As such, it may serve as a template for other, similarly inaccessible study populations.

Keywords: bycatch, delphinids, gene flow, migration, population structure

Introduction

Estimating population parameters such as effective population size, migration rate and its directionality, as well as the degree of admixture, are important in evolutionary biology. Whether individuals form part of a

single, randomly mating population or are members of different populations with varying levels of genetic isolation also has important bearings on conservation and management (Waples & Gaggiotti 2006; Frankham *et al.* 2010). Genetic data are frequently employed to determine whether, and to what extent, samples collected at different locations are part of the same population or whether they are genetically differentiated, because information on geographic separation is not usually sufficient to determine the degree of isolation (Beerli & Palczewski 2010).

Genetic differentiation among populations may be observed in cases where there has been long-term separation with low recurrent gene flow, or recent divergence with no ongoing gene flow (Nielsen & Wakeley 2001; Palsbøll *et al.* 2004). Discriminating between these two scenarios has important ramifications for conservation, as isolated populations impacted by anthropogenic stressors may require different management strategies from those that experience homogenizing effects due to gene flow (Hoelzel *et al.* 1998b; Bilgmann *et al.* 2014).

Currently, there is no general framework outlining the levels at which populations are demographically independent (Waples & Gaggiotti 2006). In migration drift equilibrium situations, assuming selective neutrality, genetic differentiation between populations is negatively correlated with the number of migrants per generation between them. Previous approaches inferred the number of migrants between populations based on the degree of genetic divergence between populations, such as Wright's F_{ST} (Wright 1931), based on a symmetric island model. However, it has been shown that these approaches are problematic, particularly as the mathematical model underlying the transformation of F_{ST} into the number of migrants per generation makes numerous assumptions, which are biologically unrealistic (e.g. Whitlock & McCauley 1999). More recently, individual-based methods have been developed that allow individuals to be assigned to populations using matching probabilities (e.g. Pritchard *et al.* 2000; Corander *et al.* 2008). Yet, these approaches are not able to estimate important population parameters, such as the directionality and extent of migration, mutation or population size, which may account for the present population structure (Palsbøll *et al.* 2007). Such information is important for assessing the impact of human activity on wildlife, but difficult to obtain in the marine environment.

Most cetacean species are impacted by human activities in at least some parts of their geographic range (Whitehead *et al.* 2000; Read *et al.* 2006). The incidental capture, or bycatch, of cetaceans in fisheries is a persistent threat to many populations (Halpern *et al.* 2007; Reeves *et al.* 2013), although knowledge of population structure and connectedness is currently lacking for many species. Gill netting, purse seining and trawling operations result in the greatest proportions of fishery-related mortalities (Read *et al.* 2006; Slooten 2013). In Australian waters, dolphins interact with prawn and fish trawling operations wherever they occur (e.g. Chilvers & Corkeron 2001; Svane 2005). Off the remote northwestern Australian coastline, dolphins regularly interact with the Pilbara Fish Trawl Interim Managed Fishery (Pilbara Trawl Fishery or 'PTF' hereafter, Jaiteh *et al.* 2013). Bycatch of a range of protected species (including dolphins, sawfish and turtles) was first highlighted in the PTF in 2002, with dolphin bycatch initially estimated at *c.* 50 individuals *per annum* (Stephenson & Chidlow 2003). An estimated minimum of 500 dolphins was caught in the 10 years from 2003 until 2012 (Allen *et al.* 2014).

The variable nature of cetacean fisheries interactions requires species- and fishery-specific approaches to bycatch mitigation (Cox *et al.* 2004, 2007). Without any prior cetacean research having been conducted in the Pilbara region, the dolphin species interacting with the PTF was previously undetermined, but assumed to be the common bottlenose dolphin (*Tursiops truncatus*), based on a limited number of length measurements and photographs. Very little is known about common bottlenose dolphins in Australian waters (Ross 2006; Allen *et al.* 2012). Bottlenose dolphins are globally widespread in tropical and temperate waters, occurring in both coastal and pelagic populations (Rice 1998; Reeves *et al.* 2002). There are three putative *Tursiops* species in Australian waters: common bottlenose and Indo-Pacific bottlenose dolphins (*T. truncatus* and *T. aduncus*, respectively) have wide distributions (Woinarski *et al.* 2014) and the Burrunan dolphin (*T. australis*; Möller *et al.* 2008; Charlton-Robb *et al.* 2011; but see Committee on Taxonomy 2015), a proposed species that is restricted to a few southeastern Australian embayments. Common bottlenose dolphins are thought to occur further offshore and generally in deeper waters than Indo-Pacific bottlenose dolphins, which inhabit near-shore areas of much of the Australian coastline, including continental islands and reefs (Woinarski *et al.* 2014). Indo-Pacific bottlenose dolphins may mix with and/or be replaced by common bottlenose dolphins in some areas, and many communities of both these species interact with trawling operations around Australia (Allen *et al.* 2014; Woinarski *et al.* 2014). Although spinner dolphins (*Stenella longirostris* sp.) also occur in northwestern Australian waters and have been subject to bycatch in commercial fisheries (Ross 2006), they are morphologically and behaviourally distinguishable from the *Tursiops* Genus, and only the bottlenose dolphin phenotype

has been reported (by skippers, crew and fisheries observers) as bycatch in the PTF (Stephenson & Chidlow 2003; Allen *et al.* 2014).

While common bottlenose dolphins may occur in deeper waters than Indo-Pacific bottlenose dolphins around Australia, the 'offshore' dolphins interacting with the PTF do so between depths of *c.* 50 and 100 m (Jaiteh *et al.* 2013; Allen *et al.* 2014). This is not deep by oceanic standards, and coastal *T. aduncus* can be found in similar depths, especially when close to islands or where there is a steep gradient adjacent to the coast (Woinarski *et al.* 2014). Thus, one cannot assume *a priori* the absence of gene flow between the two groups of dolphins ('coastal' and 'offshore'), particularly given the high levels of hybridization among delphinids (e.g. Bérubé 2009; Schaurich *et al.* 2012; Brown *et al.* 2014).

Correct species identification is critical in wildlife management, because even closely related and morphologically similar species may possess variable behavioural and life history characteristics (Wade & Angliss 1997; Boness *et al.* 2002). Here, we used an extended population genetics toolbox to investigate the species status and population genetic structure of a number of bottlenose dolphin populations off northwestern Australia (Fig. 1), the first such study in this region. We collected small tissue biopsies from: incidentally captured and free-ranging dolphins interacting with the PTF; dolphins at multiple 'shallow' coastal sites inshore of the fishery and across northwestern Australia; and, dolphins in deeper waters off the North West Cape (Fig. 1). We aimed to determine whether dolphins interacting with the PTF showed greater genetic affinities to the common bottlenose dolphin (*T. truncatus*), the Indo-Pacific bottlenose dolphin (*T. aduncus*), or other closely related delphinid taxa. Furthermore, in addition to the traditionally used combination of basic genetic summary statistics and population structure analysis, we included explicitly model-based, coalescence analyses of genetic connectedness among dolphin populations across the region. In particular, we aimed to elucidate whether there was recruitment into the PTF-associated population(s) from nearby coastal sampling sites.

Materials and methods

Sample collection and fishery characteristics

Biopsy sampling efforts were focussed at 15 sites around northwestern Australia (Fig. 1). The Pilbara Trawl Fishery is bound by longitudes of 116°E to the west and 120°E to the east, and by an approximation of the 50 m depth contour inshore and the 100 m depth contour offshore (Fig. 1). Four management areas are open to trawl fishing, representing an area of *c.* 23 000 km^2. Three vessels operated in the PTF at the time of the study and they completed between *c.* 7300 and 10 300 h of trawling *per annum* from 2010 to 2012 (Fletcher & Santoro 2013).

All dolphin biopsy samples from within the fishery were collected between *c.* 50 and 170 km offshore, in water >50 m deep and over an east west distance of *c.* 300 km. An eastern (Site 15) and a western (Site 14) cluster of samples were collected in the PTF (Fig. 1). Another three samples were collected in water *c.* 300 km to the southwest of the PTF: in deep (101 m) water offshore of the North West Cape (Site 13, 'NW Cape offshore', 114°E, Fig. 1). These three samples were included in this study to provide potential insight, albeit limited by the small sample size, into genetic connectedness of the PTF-associated dolphins to other 'offshore' populations.

Coastal biopsy sampling of bottlenose dolphins occurred at 12 sites in waters <50 m deep and within about 10 km of the coastline, extending from Useless Inlet (Site 1, 26.1°S, 113.3°E) in Shark Bay in the southwest to Cygnet Bay (Site 12, 16.5°S, 123.0°E) in King Sound in the northeast, spanning *c.* 2000 km of coastline (Fig. 1).

A total of 344 dolphin samples were collected between 2008 and 2013 (except those from Shark Bay, Sites 1 4, which were obtained between 1998 and 2013) and used for genetic analyses in this study. The subset of 68 samples of PTF-associated dolphins included three incidentally caught individuals and 65 free-ranging animals obtained during commercial fish trawling operations, on four trips to sea between 2008 and 2011. Biopsies from free-ranging dolphins were obtained using the PAXARMS remote biopsy system (Krützen *et al.* 2002) from a small (4.5 m) tender and a biopsy pole (Bilgmann *et al.* 2007) for sampling individual dolphins close to the bow or stern of trawl vessels (and a large research vessel for the three samples obtained in deeper waters offshore of the North West Cape). All 273 bottlenose dolphins sampled from the 12 coastal sites were collected from free-ranging dolphins using the PAXARMS remote biopsy system from small (5.5 m) research vessels.

Generation of genetic data

DNA was extracted from biopsy samples using the Qiagen Gentra tissue kit following the manufacturer's instructions. The extracted genomic DNA was resuspended in TE buffer (10 mM Tris, 1 mM EDTA, pH 8) and the concentration adjusted to 20 ng/μL. Sex determination was carried out by amplification of the sex specific ZFX and SRY loci using a multiplex PCR (Gilson *et al.* 1998).

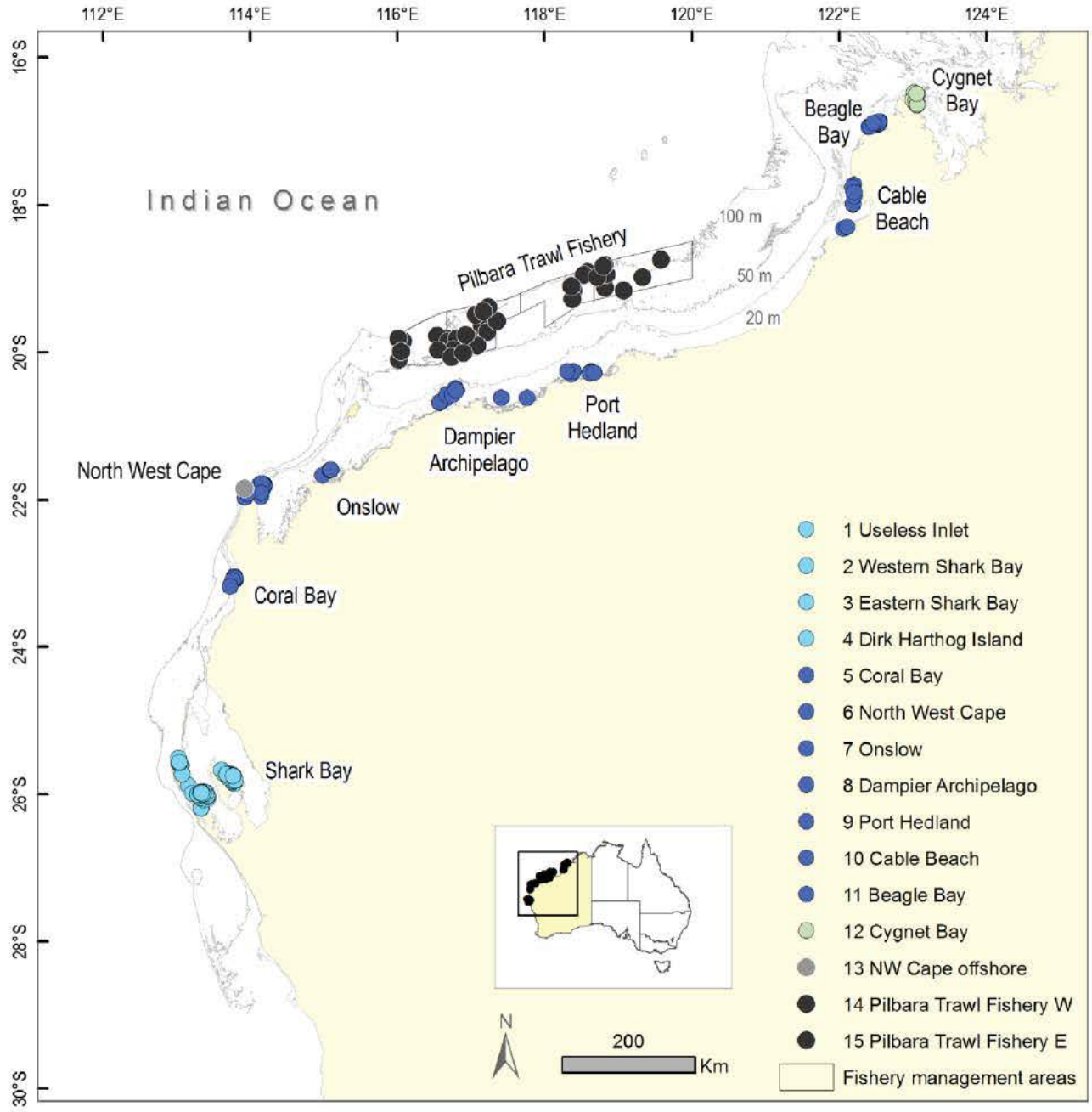

Fig. 1 Map of the sampling sites, northwestern Australia, showing the biopsy sample collection sites for: incidentally captured and free ranging dolphins associating with the Pilbara Trawl Fishery (*n* 68, black); Site 15, the 'PTF East' sampling site, was collected around 119°E, while 14, the 'PTF West' sampling site, was collected *c.* 160 km to the west, between *c.* 116°E and 117°E; dolphins in deep water off the North West Cape (*n* 3, grey); and coastal dolphins (*n* 273) from 12 sites (single circles may indicate multiple samples collected from some locations, light blue Shark Bay sites, dark blue other coastal sites, green coastal Cygnet Bay). The boundaries of the PTF management areas and the 20 m , 50 m and 100 m depth contours are also shown.

A 430-base pair part of the hypervariable region I of the mitochondrial control region (HVR-I) was amplified using primers dlp1.5 and dlp5 (Baker *et al.* 1993). PCR products were cleaned up using silica membrane spin columns (GeneElute™ by Sigma-Aldrich) and sequenced using the Cycle Sequencing Ready Reaction kit (BigDye Terminator v3.1 Applied Biosystems), based on the protocol described in Bacher *et al.* (2010), using

sequencing primer dlp 1.5. SEQUENCING ANALYSIS v5.2 and BIOEDIT v7.0.5.3 were used to visually quality control, edit and align the sequences.

Nineteen microsatellite loci were amplified using two different multiplex PCR regimes: multiplex 1 Tur4 98, Tur4 117, MK6, E12, Tur4 105, Tur4 108, Tur4 66, Tur4 111, Tur4 128 and multiplex 2 KWM12, MK3, MK5, MK8, MK9, Tur4 142, Tur4 153, Tur4 162, Tur4 80, Tur4 132 (Hoelzel *et al.* 1998a; Krützen *et al.* 2001; Nater *et al.* 2009). PCRs contained 20 ng template DNA, 5 μL 2× Multiplex PCR Master Mix (Qiagen, containing HotStar*Taq* DNA Polymerase, dNTPs and 3 mM $MgCl_2$ final concentration), between 0.05 and 0.5 μM of each primer and molecular-grade water to a final volume of 10 μL. Diluted PCR products were denatured in 10 μL HiDi formamide containing 0.07 μL of GeneScanTM500LIZ size standard (Applied Biosystems). The length of the DNA fragments was determined by running the PCR products on an ABI 3730 DNA Sequencer (Applied Biosystems) and analysing the output files using GENEMAPPER v4.0. We independently amplified and scored 20 randomly selected individuals to estimate error rate for the microsatellite scoring. Our error rate was determined to be 0.0039 (three scoring differences in 760 alleles).

Population structure and gene flow

Population structure and genetic connectedness among sampling localities were inferred using both summary statistics and individual-based approaches based on microsatellite data. Genetic variation within sampling sites was estimated by calculating the number of alleles and effective alleles, observed (H_O), expected (H_E) and unbiased expected heterozygosity (UH_E) in GENALEX v6.5 (Peakall & Smouse 2012). Tests for departure from Hardy Weinberg equilibrium (HWE) and the occurrence of linkage disequilibrium and null alleles were carried out for each sampling site in GENEPOP v4.2.1 (Rousset 2008), with Bonferroni-corrected significance levels (Rice 1989). Estimates of F_{ST} (Weir & Cockerham 1984) and Jost's D (Jost 2008) were calculated in GENEPOP and GENODIVE (Meirmans & Van Tienderen 2004), respectively.

The software package STRUCTURE v2.3.3 (Pritchard *et al.* 2000) was used to determine the genetic structure and number of genetic clusters in our data set. In particular, we were interested in the levels of genetic connectedness among the PTF-associated population(s) and the 12 coastal dolphin sampling localities. The STRUCTURE algorithm divides sampled individuals into a number of clusters (K) independent of locality information by minimizing deviations from Hardy Weinberg and linkage equilibrium in each cluster. The software uses a Markov chain Monte Carlo (MCMC) procedure to estimate P(X|K), the posterior probability that the data fit the hypothesis of K clusters.

Three different STRUCTURE analyses were conducted. The length of the burn-in period was set to 10^5, followed by 10^6 MCMC steps. For each K (the maximum number of K for each analysis was the number of sampling locations for the respective analysis), the analysis was run 10 times. The first, global analysis involved all samples and used an admixture model with correlated allele frequencies and no prior information. For the two subsequent analyses, we chose the 'Locprior' model, which improves clustering when the signal is weak without spuriously inferring structure, if absent (Hubisz *et al.* 2009). The second analysis was carried out on PTF individuals only, while the third analysis incorporated only the 12 coastal populations. As the P(X|K) estimator has been shown to overestimate K, as it frequently plateaus at higher values than biologically meaningful estimates of K, we also calculated the ΔK statistic (Evanno *et al.* 2005). This provides a very conservative estimate of K only at the highest biological level and was performed using the software STRUCTUREHARVESTER (Earl & vonHoldt 2012).

In addition, a factorial correspondence analysis projecting all genotypes on the factor space, which is defined by the similarity of their allelic states, as implemented in GENETIX v4.05.2 (Belkhir *et al.* 2004), was used to visualize the degree of dissimilarity among sampling sites.

Migration patterns and gene flow among the PTF population and selected coastal populations were inferred based on two coalescence modelling approaches. The first approach was implemented in MIGRATE-N v3.6.4 (Beerli & Felsenstein 2001; Beerli 2006), which is based on an equilibrium island model to estimate genetic diversity of each defined population and all pairwise migration rates between these. This analysis was based solely on microsatellite data as the software does not implement a correction for differing inheritance modes, that is, mitochondrial DNA (mtDNA) vs nuclear DNA. In order to reduce the number of parameters in our models to arrive at a computationally and statistically tractable analysis, some relevant sampling sites were pooled into three populations (as identified in our STRUCTURE analysis, see Results): Pilbara Trawl Fishery (Sites 14 15), Shark Bay (Sites 1 4) and 'Other Coastal' populations (Sites 5 11; Cygnet Bay was excluded because our STRUCTURE and factorial correspondence analyses revealed at least some Cygnet Bay individuals to be genetically different). Four different models (Table 2) constraining the presence, directionality and amount of gene flow among the three pooled sampling sites were defined. Model 1 allowed full migration between all population pairs (full model).

One cannot define a model that sets migration among PTF and all other populations to nought because, under such circumstances, coalescence trees could not be calculated and general assumptions of the MIGRATE-N approach were violated. Therefore, model 2 allowed only very limited gene flow from and to PTF (≈ nought migration, but sufficient to match MIGRATE-N's needs with regard to coalescence trees). This effectively rendered the PTF population isolated from both Shark Bay and Other Coastal populations, while it allowed full migration between Shark Bay and Other Coastal populations (low migration PTF model). In model 3, gene flow from the PTF population into the Shark Bay and Other Coastal was allowed, but not vice versa. In model 4, gene flow from Shark Bay and Other Coastal populations to the PTF was allowed, but not vice versa. Convergence was achieved by running each model for more than 80 000 CPU hours, parallelized over 240 CPUs. We used 50 independent, replicate runs, each with its own burn-in and heating scheme, to later join the results (c.f., Hartmann *et al.* 2013). We regard the emergence of clear, unimodal posterior distributions across all these replicates as a strong indicator of convergence.

The run parameters for MIGRATE-N were as follows: for Θ (population size parameter, scaled to mutation rate) and *M* (migration rate parameter), a uniform prior was used. The prior range for Θ was set to 0 10 (mean 5; Δ 1; 20 000 bins) and for *M* 0 100 (mean 50; Δ 10; 20 000 bins). Mutation rates of loci were allowed to vary. Five hundred coalescent samples were recorded per replicate, one every 100 iterations, thus sampling 25 000 (50 × 500) parameter values from chains comprising a total of 2 500 000 iterations. A static heating scheme (4 chains with temperatures 1 000 000; 3; 1.5; 1) and a burn-in of 200 000 steps were applied to each replicate. Model comparisons were carried out using marginal likelihoods calculated using the thermodynamic integration ('Bezier') in MIGRATE-N (Beerli & Palczewski 2010). The estimated mutation-scaled migration parameter *M* was translated into the effective number of immigrants per generation (*Nm*), as detailed elsewhere (Jonker *et al.* 2013; Kraus *et al.* 2013).

The second coalescence approach to assess migration patterns and gene flow between the PTF population and selected coastal populations was implemented in IMA2 (Nielsen & Wakeley 2001; Hey & Nielsen 2007; Hey 2010). This approach is based on an isolation-with-migration (IM) model (allows for lack of gene flow, as opposed to MIGRATE-N) and uses Metropolis-coupled Markov chains to approximate posterior distributions of population size, gene flow and divergence time. Similar to our MIGRATE-N analysis, sampling sites were pooled into three populations (as identified in our STRUCTURE analysis, see Results): Pilbara Trawl Fishery (PTF, Sites 14 15), Shark Bay (Sites 1 4) and Other Coastal (Sites 5 11, i.e. Cygnet Bay excluded). In contrast to the MIGRATE-N analysis, however, we also included mtDNA data, because the software has a built-in ability to weigh across different inheritance modes. As IMA2 is slow for large multilocus data sets, we randomly selected 30 individuals from each of the three populations.

For the IMA2 analysis, we used uniform priors for divergence times and population sizes. For migration rates, exponential priors may be more informative when actual rates of gene flow are very low or nought (Runemark *et al.* 2012). However, among our three populations, gene flow might be substantial among the Other Coastal and Shark Bay populations. Thus, we also used uniform priors for migration rates. Mutation rates were set to 4.8×10^{-8} (range 3.1×10^{-8} to 6.9×10^{-8}; Oremus *et al.* 2007) mutations/year for mtDNA and 1.5×10^{-5} (Brohede & Ellegren 1999) for all microsatellite loci. Upper limits for divergence time were set to $t = 30$, population size Θ 150 and migration rate *M* 50. The latter value appears high, but several initial runs (burn-in period of 20 000 and run length of 100 000) had shown that the parameter estimate of *M* between the Other Coastal and Shark Bay populations was very high. We carried out several independent runs. In each run, to ensure adequate mixing of the Markov chain, we used Metropolis coupling of 60 independent heated chains (Geyer 1992). Burn-in took place until stationarity was reached by assessing burn-trend plots for each run. The most heated chain had a heating factor of 0.9, with other chains having heating values between 1 and 0.9. As suggested by Hey (2010), stationarity for each run was evaluated by assessing autocorrelations of splitting time terms, the absence of trends in splitting time trend plots and by the degree of similarity between parameter estimates from genealogies generated during the first and the second half of the run.

To obtain estimates of magnitude and direction of contemporary gene flow between pairs of pooled populations, we used the software BAYESASS, v3.0.3 (Wilson & Rannala 2003). This approach uses an MCMC algorithm to estimate the posterior probability distribution of the proportion of migrants between pairs of populations without assuming genetic equilibrium. We used the same three population classifications as for the MIGRATE-N and IMA2 analyses, plus a fourth (Cygnet Bay, as BAYESASS accommodates for a larger number of populations with a moderate number of markers), and conducted five independent runs for 10 000 000 generations, while discarding the first 1 000 000 generations as burn-in. Mixing parameters for the five runs were *m* 0.3, *a* 0.5 and *f* 0.5.

Finally, to learn more about coastal dolphin population structure, we conducted an isolation-by-distance analysis (Wright 1943) for all coastal populations, that is Shark Bay (Sites 1 4) and Other Coastal populations (Sites 5 12, i.e. including Cygnet Bay), based on our microsatellite data. Geographic distances between each sampling site were measured in the most direct line through the water using ArcGIS, v. 9.2 (ESRI), where the centroids for each population were estimated by including each sample taken at a particular site. We tested for a decrease in genetic similarity (based on F_{ST}) with increasing geographic distance, using a Mantel test implemented in IBDWS, v.3.23 (Jensen *et al.* 2005). Significance was evaluated by 10 000 randomizations.

Phylogenetic analyses

The mtDNA sequence alignment was trimmed to the shortest sequence, and part of the 5′ tRNA sequence was removed, resulting in a 399-bp fragment. Identical haplotypes were collapsed using Dambe v5.0.72 (Xia & Xie 2001). We used a general time-reversible model with gamma-distributed rate variation across sites and a proportion of invariable sites, as implemented in MrBayes v3.2, thereby sampling across the substitution model space in the Bayesian MCMC analysis itself (Huelsenbeck *et al.* 2004). Parameters for the MrBayes run were four chains running for 10 000 000 generations, with a sampling frequency of 1000 and a burn-in of 2500 data points. Consensus trees were displayed and printed using FigTree v1.1.2 (http://tree.bio.ed.ac.uk/).

To assess phylogenetic affiliations of the PTF-associated bottlenose dolphins with other delphinids, previously published HVR-I sequences from the following species and regions were included in the analysis: common (*T. truncatus*) and Indo-Pacific (*T. aduncus*) bottlenose dolphins, principally from Chinese and Indonesian waters (Wang *et al.* 1999), as well as the recently delineated Burrunan dolphin from Victoria, Australia (*T. australis*; Charlton-Robb *et al.* 2011) and Fraser's dolphins (*Lagenodelphis hosei*; Caballero *et al.* 2008; Table S1, Supporting information). We also included *T. aduncus* samples from coastal southeastern Australia (Möller & Beheregaray 2001; Möller *et al.* 2008; Wiszniewski *et al.* 2010). The tree was rooted with an Atlantic white-sided dolphin (*Lagenorhynchus acutus*; Cipriano 1997) sequence as an out-group (Table S1, Supporting information).

Results

Within each sampling site, all 19 microsatellite loci were in Hardy Weinberg equilibrium. No significant linkage disequilibrium or consistent occurrence of null alleles in markers across all populations were observed. Allelic diversity and heterozygosity values were generally higher for the PTF samples compared with coastal sampling sites (Table S2, Supporting information). The pairwise F_{ST} values obtained from microsatellite data were small (generally <0.06), but significant among almost all sampling sites (Table 1). The highest values (generally >0.20) were observed for all pairwise comparisons between offshore and coastal sampling sites (Table 1). This suggests a longer period of isolation between offshore and coastal populations than among different coastal sampling sites. Pairwise values for Jost's *D* were generally larger than F_{ST} values. In particular, pairwise comparisons between PTF and coastal populations were on average 2 3 times larger for Jost's D than for F_{ST}, suggesting that F_{ST} underestimates divergence (Whitlock 2011).

For the global data set containing all samples, the Evanno method identified that K 2 clusters was the most likely scenario. The Structure analysis illustrated a clear pattern of genetic differentiation between the offshore (both the PTF and NW Cape offshore) and all coastal sampling sites (Fig. 2a). For higher K values for the global data set, visual inspection revealed four clusters: (i) the four Shark Bay coastal sites, (ii) all coastal sites from Coral Bay to Beagle Bay, (iii) coastal Cygnet Bay and (iv) the NW Cape offshore and PTF (Fig. 2a).

When only PTF samples were considered, K 1 had the highest probability, suggesting no genetic substructuring within the PTF. There was also no indication of any admixed individuals within the PTF, which could have been conceivable given the occurrence of Fraser's dolphin haplotypes (see below) in the PTF data set. When only coastal samples were considered (Fig. 2b), Shark Bay sites formed a distinct cluster from all other coastal sites, which was also supported by the Evanno method (ΔK 2). At K 3 and higher, samples from Cygnet Bay became distinct, but the remaining coastal populations formed one cluster.

The factorial correspondence analysis based on 19 microsatellite loci (Fig. 3) strongly supported the Structure results. Samples from the PTF formed a single distinct cluster compared with all other samples, including NW Cape offshore. Among the coastal sites, the four Shark Bay sites in the southwest were clearly distinct from other sites across the northwest, while Cygnet Bay was distinct in the northeast. All other coastal sites could not be distinguished from each other (Fig. 3). An isolation-by-distance analysis on only coastal samples revealed a highly significant correlation (r 0.48, $P < 0.01$) among all individual coastal sites (Fig. 4).

Based on the Structure results, we pooled most sampling localities into three 'populations' to analyse

Table 1 Pairwise F_{ST} (above) and Jost's D (below the diagonal) values between sampling sites

Sampling Site	Useless Inlet	Western Shark Bay	Eastern Shark Bay	Dirk Hartog Island	Coral Bay	North West Cape	Onslow	Dampier Archipelago	Port Hedland	Cable Beach	Beagle Bay	Cygnet Bay	NW Cape offshore	PTF West	PTF East
1 Useless Inlet		0 006	**0.031**	**0.038**	**0.059**	**0.042**	**0.025**	**0.041**	**0.033**	**0.060**	**0.046**	**0.065**	**0.251**	**0.279**	**0.263**
2 Western Shark Bay	0 025		**0.028**	**0.018**	**0.045**	**0.032**	**0.018**	**0.043**	**0.039**	**0.060**	**0.047**	**0.059**	**0.234**	**0.272**	**0.254**
3 Eastern Shark Bay	0 106	0 003		**0.036**	**0.054**	**0.066**	**0.044**	**0.053**	**0.039**	**0.060**	**0.058**	**0.082**	**0.259**	**0.279**	**0.263**
4 Dirk Hartog Island	0 225	0 041	0 004		**0.047**	**0.052**	**0.033**	**0.042**	**0.038**	**0.067**	**0.036**	**0.062**	**0.230**	**0.263**	**0.243**
5 Coral Bay	0 123	0 145	0 173	0 186		**0.015**	0 008	**0.020**	**0.025**	**0.028**	0 005	**0.051**	**0.233**	**0.264**	**0.244**
6 North West Cape	0 117	0 009	0 016	0 003	0 040		0 009	**0.020**	**0.032**	**0.040**	**0.022**	**0.067**	**0.285**	**0.291**	**0.276**
7 Onslow	−0 097	−0 126	−0 085	−0 004	0 076	−0 038		−0 006	0 017	0 006	0 009	**0.040**	**0.192**	**0.252**	**0.230**
8 Dampier Archipelago	0 104	0 001	−0 002	−0 004	0 063	−0 026	−0 097		**0.008**	0 010	**0.012**	**0.051**	**0.231**	**0.268**	**0.249**
9 Port Hedland	0 081	0 088	0 130	0 149	−0 059	0 023	0 001	0 032		**0.028**	**0.012**	**0.044**	**0.238**	**0.264**	**0.248**
10 Cable Beach	−0 016	0 101	0 107	0 253	0 178	0 175	−0 105	0 124	0 142		**0.036**	**0.057**	**0.199**	**0.253**	**0.233**
11 Beagle Bay	0 145	0 107	0 168	0 151	−0 051	0 021	0 093	0 062	−0 037	0 264		**0.047**	**0.230**	**0.256**	**0.233**
12 Cygnet Bay	0 250	0 214	0 225	0 188	−0 038	0 067	0 193	0 105	−0 001	0 324	−0 030		**0.199**	**0.247**	**0.231**
13 NW Cape offshore	0 491	0 774	0 685	0 873	0 706	0 835	0 480	0 721	0 685	0 235	0 892	0 861		**0.070**	**0.063**
14 PTF West	0 473	0 686	0 643	0 791	0 613	0 721	0 492	0 664	0 609	0 319	0 743	0 735	0 157		0 002
15 PTF East	0 418	0 626	0 574	0 721	0 563	0 661	0 396	0 589	0 551	0 246	0 701	0 692	0 092	−0 041	

Significant F_{ST} values (after Bonferroni correction, Rice 1989) are given in bold The shaded area represents offshore–coastal population comparisons

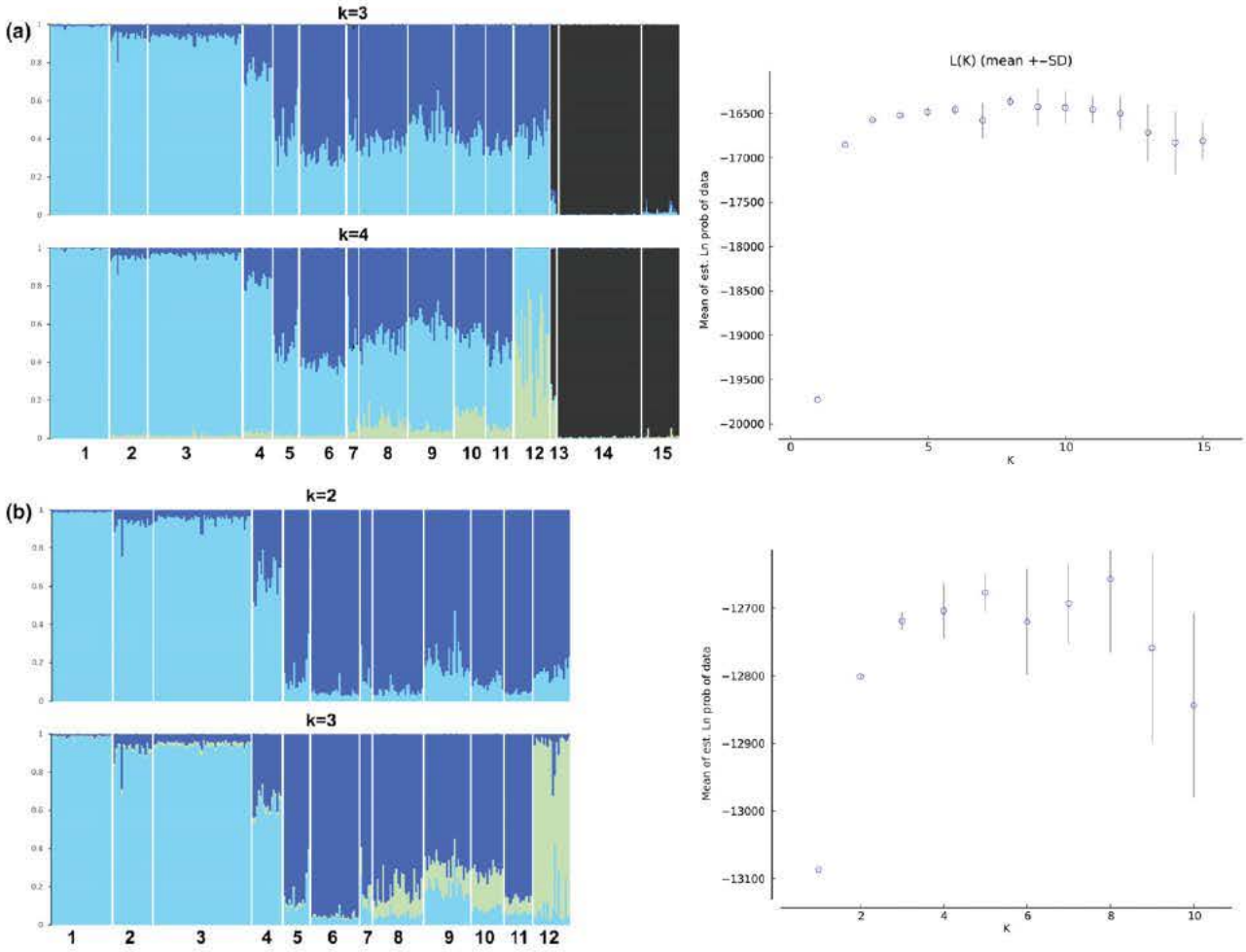

Fig. 2 Structure plots (each column representing assignment probability of an individual dolphin, with sampling sites separated by a white line) and log likelihoods for different number of clusters, *K*: (a) Full data set including all northwestern Australian samples (*n* 344). (b) Coastal samples only (*n* 273). The sampling site numbers correspond to their geographic location from the southwest to the northeast (coastal and then offshore) as in Fig. 1: 1 Useless Inlet; 2 Western Shark Bay; 3 Eastern Shark Bay; 4 Dirk Hartog Island; 5 Coral Bay; 6 North West Cape; 7 Onslow; 8 Dampier Archipelago; 9 Port Hedland; 10 Cable Beach; 11 Beagle Bay; 12 Cygnet Bay; 13 NW Cape offshore; 14 PTF West; 15 PTF East.

migration patterns among the combination of: (i) all four Shark Bay coastal sites ('Shark Bay'), (ii) all other coastal sites, other than Cygnet Bay ('Other Coastal') and (iii) PTF West and East into a single population ('PTF').

Our model comparisons showed a clear lack of migration into the PTF population from any of the coastal populations (Table 2). The model with the lowest support was that which allowed free migration among all populations (Table 2). Thus, our results suggest strongly that the PTF population is reproductively isolated from coastal populations, with no recruitment of dolphins into the PTF population from nearby coastal areas.

We based our parameter estimates of Θ (a mutation-scaled measure for population size) and *Nm* (the head count of effective migrants per generation) on the model that allowed estimation of Θ for all populations. As expected, Θ was highest for the pelagic PTF population (Θ = 6.37, 95% CI 5.60 7.26). The coastal populations had smaller Θ values (Shark Bay Θ = 0.78, 95% CI 0.53 1.00; Other Coastal Θ = 2.90, 95% CI 2.48 5.29). As there was no gene flow from the PTF to any of the coastal populations, we only report *Nm* estimates between the latter. The *Nm* estimate differed significantly from nought in both cases, with *Nm* values from Shark Bay to Other Coastal populations being higher than vice versa (SB → OC: *Nm* 4.31, 95% *CI* 3.70 7.89; OC → SB: *Nm* 0.21, 95% CI 0.14 0.26). Importantly, in all models, regardless of their level of support, the *Nm* parameter estimates concerning migration into the PTF population were always small and confidence

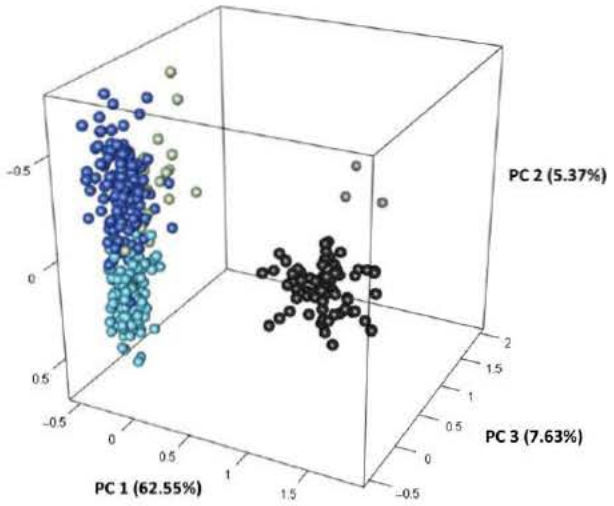

Fig. 3 Three dimensional representation of a factorial correspondence analysis projecting all sampled individuals of northwestern Australian bottlenose (*Tursiops* spp.) dolphins on the factor space. The factor space is defined by the similarity of allelic states, in order to visualize the degree of dissimilarity among sampling sites. As per Figs 1 and 2: black/grey individuals sampled in deeper (>50 m) waters in the PTF/NW Cape offshore; blue/green individuals sampled in shallower (<50 m), coastal sites.

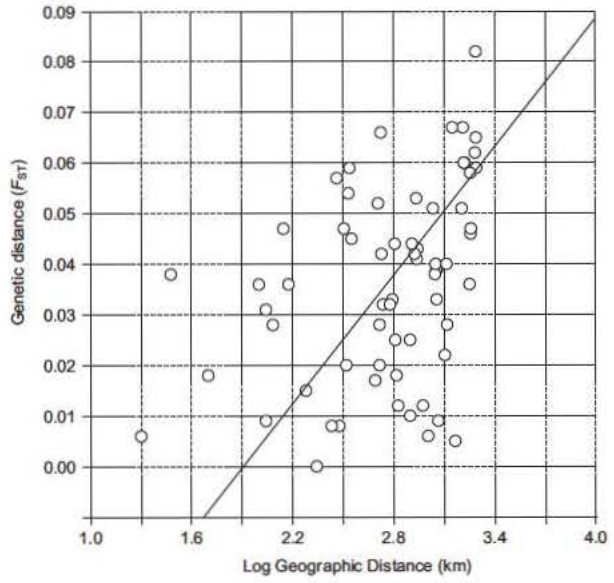

Fig. 4 Isolation by distance plot of correlation between genetic (F_{ST}) and geographic (km) distance for all coastal sampling locations (Sites 1 12), which is highly significant (r 0.482, P 0.0011).

intervals included 0, providing further evidence of the lack of recruitment of dolphins into the PTF population from nearby coastal areas. Result files for each model are available online as supplementary material.

Our IMa2 analyses corroborated those obtained by Migrate-n. Effective population size was largest for the PTF population (Fig. 5, Θ = 2.46, 95% CI 1.57 3.5) and smaller for the coastal populations (Shark Bay Θ = 0.44, 95% CI 0.04 1.09; Other Coastal Θ = 1.65, 95% CI 0.56 4.56). Migration rate parameters were only significant between Shark Bay and Other Coastal populations (log-likelihood ratio (LLR) 5.45, $P < 0.01$) and vice versa (LLR 2.87, $P < 0.05$, Fig. 5). The 95% confidence intervals of all migration parameters between the PTF and the two coastal populations included 0 and were not significant (Fig. 5), providing further evidence for the lack of gene flow between the PTF and all coastal populations.

The results from the Migrate-n and IMa2 analyses were corroborated by our findings based on BayesAss (Table 3). We could not detect any significant migration from the coastal populations into the PTF population and vice versa. In general, the proportion of detected migrants within each population (other than Cygnet Bay, which received about 28% of migrants from the Other Coastal population) was small, and the 95% confidence interval included 0 in almost every comparison. These findings suggest strongly that there is no, or at most only extremely low, migration between the PTF, and the Shark Bay and Other Coastal populations.

Table 2 Comparisons of four different migration models used in Migrate n. For model comparisons, we pooled sampling sites into three populations (see Materials and methods)

Model	Populations and model parameters			Bezier approximation score (BAS)	ΔBAS
	SB	OC	PTF		
Full	***	***	***	359 466	133 001
Low migration PTF	**c	**c	cc*	237 198	10 733
PTF → SB/OC	***	***	00*	226 465	0
SB/OC → PTF	**0	**0	***	260 614	34 149

SB Shark Bay (Sites 1 4), OC Other Coastal (Sites 5 11), PTF Pilbara Trawl Fishery (Sites 14 15). In each of the migration models, nine parameters, that is, migration rates between populations, were considered. Asterisks indicate that migration rates were estimated by Migrate n. In some migration models, we set the migration rate among certain populations to nought (0), or allowed only a fixed, low (c) migration rate of 0.001 (see Materials and methods). Parameters 1 3 indicate migration rate into the SB populations from the SB, OC and PTF populations, parameters 4 6 indicate migration rate into the OC population from the SB, OC and PTF populations, and parameters 7 9 indicate migration rate into the PTF population from the SB, OC and PTF populations. Model scores are given by Bezier approximation and differences between models highlighted in column ΔBAS.

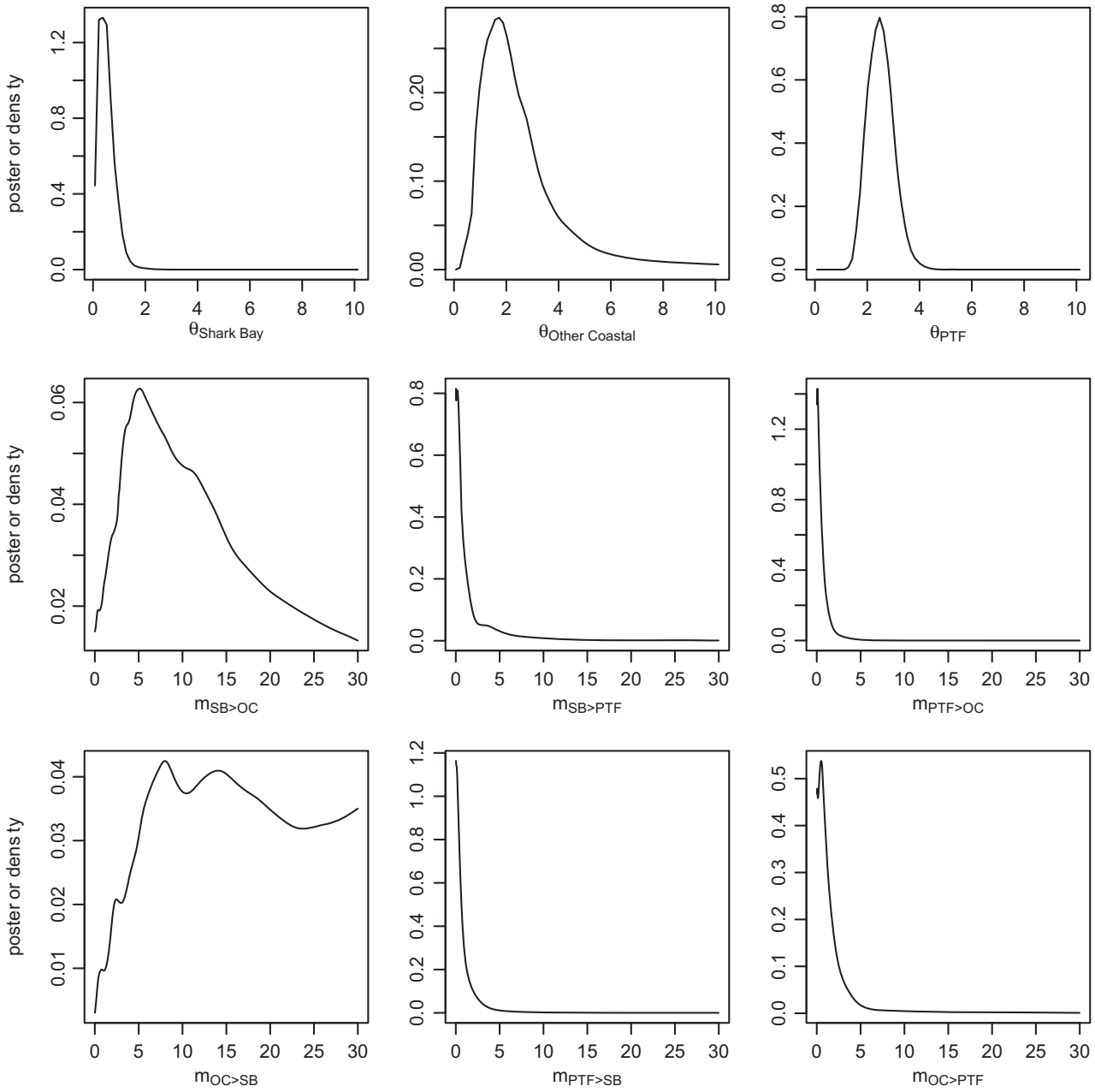

Fig. 5 Posterior density distributions for IMA2 simulations. Θ = relative effective population size, m migration rate, PTF Pilbara Trawl Fishery, SB Shark Bay, OC Other Coastal.

The phylogenetic analyses based on mtDNA revealed some unexpected patterns. We identified 17 unique haplotypes among all individuals collected from within the two sampling sites in the PTF (Sites 14 and 15, Fig. 1), as well as those collected in deep water offshore of the North West Cape (Site 13). These haplotypes formed a well-supported, monophyletic clade with the common bottlenose dolphin. Within this clade, however, clear resolution was lacking (Fig. 6). The haplotype of six individuals sampled within the fishery formed a well-supported monophyletic clade (posterior probability of 0.97) with Fraser's dolphin haplotypes (Fig. 6), an unexpected result that is discussed below. While at-sea differentiation among delphinids can be difficult, all observations and photographs taken during offshore field trips were of the common bottlenose dolphin phenotype. All of the bottlenose dolphins sampled in the coastal regions of northwestern Australia formed a highly supported monophyletic clade (posterior probability of 1.00) with other Indo-Pacific bottlenose dolphins (*T. aduncus*; Figs 1, 6).

Table 3 Mean posterior distribution values (95% CI) of fraction of individuals in population *i* that are migrants derived from popu lation *j* (per generation) among four combined populations as determined by BayesAss. 95% confidence intervals smaller than 0 and larger than 1 were rounded to the nearest integer

From/to	Shark Bay	Other Coastal	Cygnet Bay	PTF
Shark Bay	0.982 (0.963 1.000)	0.013 (0.000 0.031)	0.003 (0.000 0.008)	0.003 (0.000 0.008)
Other Coastal	0.034 (0.000 0.071)	0.961 (0.924 0.998)	0.003 (0.000 0.007)	0.003 (0.000 0.007)
Cygnet Bay	0.022 (0.000 0.059)	0.284 (0.235 0.334)	0.681 (0.654 0.707)	0.013 (0.000 0.037)
PTF	0.005 (0.000 0.014)	0.005 (0.000 0.014)	0.005 (0.000 0.014)	0.986 (0.971 1.000)

Discussion

Lack of contemporary and historic gene flow between fishery-impacted and coastal dolphins

All our analyses based on nuclear microsatellite data suggest strongly that the bottlenose dolphins sampled in the offshore, pelagic environment (*Tursiops truncatus*) are genetically isolated from those sampled coastally (*T. aduncus*). Both the Structure and factorial correspondence analyses revealed four clusters of individuals that were geographically separated (one offshore and three coastal 'populations'). Similarly strong patterns of segregation have been reported in other small cetaceans. For example, Perrin *et al.* (2011) used cranial osteological differentiation to support previous assertions, based on molecular data, for the existence of coastal and offshore forms of common bottlenose dolphins (*T. truncatus*) in Californian waters. Also, false killer whale (*Pseudorca crassidens*) populations sampled offshore in the central and eastern Pacific were recently differentiated from those that are resident and island-associated around the Hawai'ian Archipelago (Martien *et al.* 2014).

Our Structure and factorial correspondence analyses, however, did not reveal whether the genetic isolation between the PTF and coastal populations is due to historic cessation of gene flow (i.e. reproductive isolation followed by speciation), or recent divergence. All analyses pertaining to migration rates revealed an absence of gene flow from any coastal population into the PTF population and vice versa, strongly suggesting that the bottlenose dolphin population that is subject to incidental capture in the PTF is genetically isolated from all the adjacent, coastal dolphins and does not recruit from these coastal dolphin populations. Furthermore, we found no evidence of hybridization between the pelagic common bottlenose dolphins (*T. truncatus*) and the coastal Indo-Pacific bottlenose dolphins (*T. aduncus*). This finding is consistent with that for these two species in Chinese waters, which, despite some areas of overlap in distribution, were found to be reproductively isolated and did not share mtDNA haplotypes (Wang *et al.* 1999; Yang *et al.* 2005).

In our study, the pelagic common bottlenose dolphins showed less genetic substructuring than the coastal Indo-Pacific bottlenose dolphins sampled across a similar geographic distance. Furthermore, the mutation-scaled, effective population sizes (Θ) of common bottlenose dolphins were much larger than those of the Indo-Pacific bottlenose dolphin population in Shark Bay and the combined coastal populations. These results were to be expected, given the more complex coastal habitat, environmental and social barriers to gene flow, and limited dispersal (Krützen *et al.* 2004; Frère *et al.* 2010), as well as the propensity for coastal *Tursiops* of both species to adapt rapidly to local habitats (e.g. Hoelzel *et al.* 1998b; Sellas *et al.* 2005; Wiszniewski *et al.* 2010). Common bottlenose dolphins of open, pelagic environments are capable of long-distance movements: for example, Wells *et al.* (1999) documented travel distances of *c.* 2000 and >4000 km in < 50 days by two satellite-tracked individuals off the east coast of the United States. Furthermore, Quérouil *et al.* (2007) found no genetic differentiation among common bottlenose dolphins from the Azores, Madeira and other offshore areas of the northeast Atlantic, suggesting that they form a large, pelagic population. The lack of baseline data on Australian common bottlenose dolphins means it is not possible to assess whether the population in the PTF region is an isolated unit or forms part of a large, pelagic population (Ross 2006). The relatively large Θ supports the latter view, but our factorial correspondence analysis revealed some segregation between common bottlenose dolphins in the PTF and those of the North West Cape (Fig. 3), and photographic evidence shows that at least a proportion of the PTF-associated population display long-term fidelity to foraging around the trawlers (Allen 2015).

Pelagic, common bottlenose dolphins of northwestern Australia

Most dolphins associated with the PTF, as well as those sampled in deeper (>50 m) waters off the North West Cape, exhibited haplotypes that form a monophyletic clade with those previously published for common

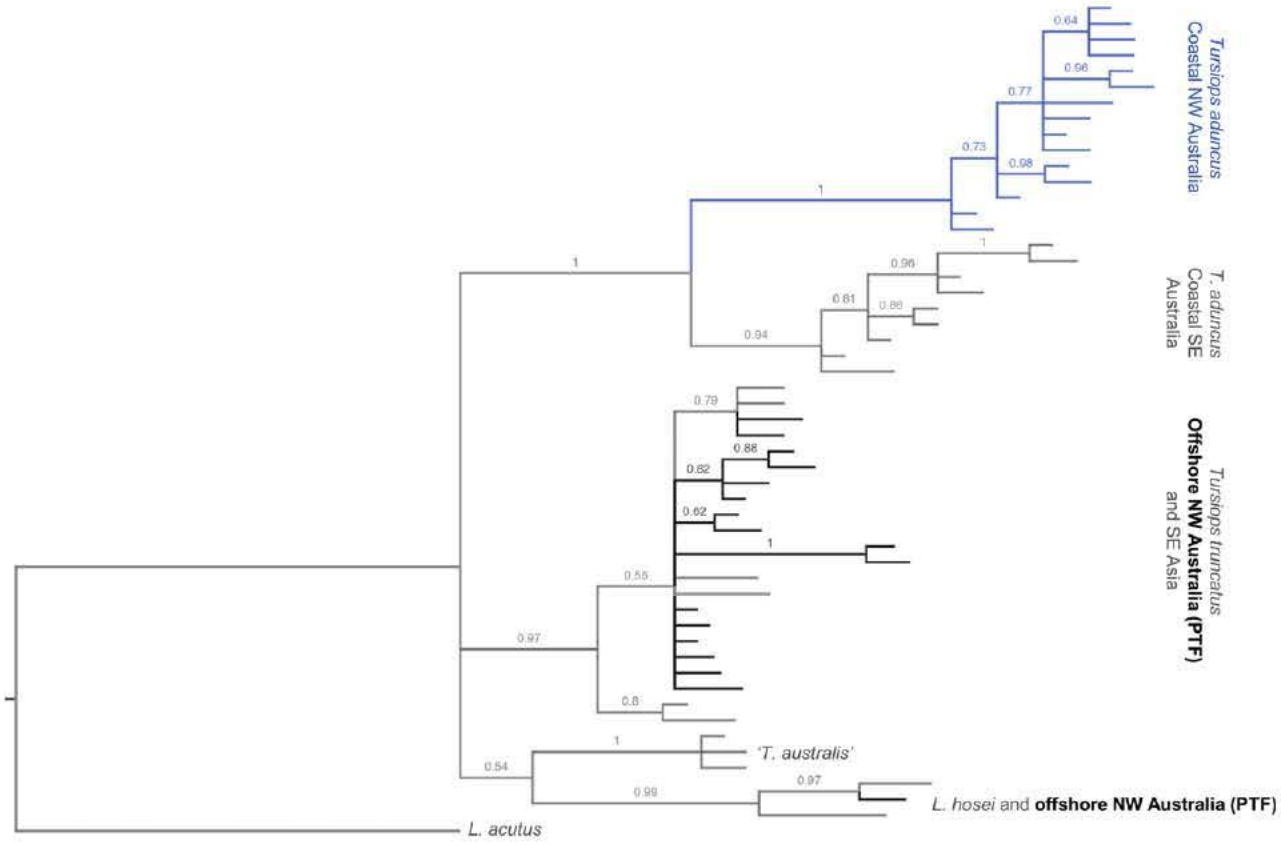

Fig. 6 Phylogenetic relationships of offshore northwestern Australian (Pilbara Trawl Fishery and North West Cape) dolphin mtDNA haplotypes and coastal northwestern Australian dolphin mtDNA haplotypes compared to relevant delphinids, based on an alignment of 399 base pairs of the hypervariable region I. Node labels are posterior probabilities. Taxa in black branches are PTF associated samples from this study. Coastal samples from this study (blue) formed a reciprocally monophyletic clade to previously published *Tursiops aduncus* from coastal southeast Australia. Taxa in grey branches are those from other studies.

bottlenose dolphins (*T. truncatus*) from Chinese and Indonesian waters. Until the current study, the Chinese and Indonesian haplotypes were the only available reference samples for *T. truncatus* in this region, despite the fact that they are globally widespread in both coastal and pelagic populations (Rice 1998; Reeves *et al.* 2002).

Bottlenose dolphins are polytypic, with two species recognized based on both genetics and morphology: *T. truncatus* and *T. aduncus* (Rice 1998; Wang *et al.* 1999, 2000a,b), and a third species proposed recently: *T. australis* (Möller *et al.* 2008; Charlton-Robb *et al.* 2011). The number of species/subspecies in the complex, however, remains to be resolved (e.g. Natoli *et al.* 2004), with the Society for Marine Mammalogy currently recognizing only *T. truncatus* and *T. aduncus* (Committee on Taxonomy 2015). All three putative species are present in Australian waters, with *T. truncatus* generally thought to occur further offshore and in deeper waters than *T. aduncus* (Ross 2006), a pattern confirmed for northwestern Australia in this study.

The use of the mitochondrial control region for phylogenetic species identification has also proven effective in a range of other studies for closely related delphinids (e.g. Rosel *et al.* 1994; Möller & Beheregaray 2001; Ross *et al.* 2003; Beasley *et al.* 2005). This marker system also has limitations, however, and its usefulness for species identification depends on the evolutionary distinctiveness of the taxa in question. In studies attempting to elucidate the evolutionary relationships among the Delphininae, the use of a single mitochondrial gene has provided limited resolution, due to high levels of intraspecific variation and low interspecific differences (Kingston *et al.* 2009; Viricel & Rosel 2012). Kingston *et al.* (2009) found that data from amplified fragment length polymorphisms (AFLPs), representing many nuclear genes, gave better resolution. However, even the use of genomewide multilocus data sets such as this, and others (Xiong *et al.* 2009; McGowen 2011; Zhou *et al.* 2011), has not been able to resolve relationships unambiguously within the Delphininae, which is thought to have undergone a recent and rapid radiation

(Kingston *et al.* 2009; Vilstrup *et al.* 2011; Hassanin *et al.* 2012).

In our study, both *T. truncatus* and *T. aduncus* formed well-supported monophyletic clades, as has been documented elsewhere (e.g. Möller & Beheregaray 2001; Moura *et al.* 2013). Most individuals from within the PTF, and elsewhere offshore, fell within the *T. truncatus* clade, providing strong evidence that it is predominantly common bottlenose dolphins associating with the fishery. These results were corroborated by the lack of both historic and contemporary gene flow between the PTF-associated common and coastal Indo-Pacific bottlenose dolphins, identified from our microsatellite data and two independent approaches to estimate gene flow.

Unexpectedly, some offshore individuals exhibited a haplotype that shares a close affinity to Fraser's dolphin haplotypes. Fraser's dolphins occur primarily in waters deeper than 1000 m (Reeves *et al.* 2002). They are rarely found in shallow waters or near-shore environs, and field guides and texts report Fraser's dolphins in mixed-species assemblages only with false killer, melon-headed (*Peponocephala electra*) and sperm (*Physeter macrocephalus*) whales, as well as Risso's (*Grampus griseus*), pan-tropical spotted (*Stenella attenuata*) and striped (*S. coeruleoalba*) dolphins (Carwardine 1995; Reeves *et al.* 2002; Dixon 2008; Jefferson *et al.* 2008). Fraser's dolphins have not been observed in mixed assemblages with bottlenose dolphins, nor would they be expected in the relatively shallow waters (50 100 m deep) in which the PTF operates.

There are three plausible explanations for the occurrence of the Fraser's dolphin haplotypes among the PTF-associated dolphins. First, both *T. truncatus* and *L. hosei* may have been present in the groups of dolphins that were sampled. However, a careful re-examination of all photographs taken in the field revealed only the bottlenose dolphin phenotype, and the STRUCTURE analysis did not reveal any admixed individuals within the PTF. Second, incomplete lineage sorting may have led to the observed pattern. Under a neutral model of evolution, the stochastic lineage sorting leading to reciprocal monophyly proceeds more slowly in large or rapidly diverging populations. In many groups of species with large population size, such as the Delphinidae (Rice 1998; McGowen 2011), genomes will have mixed support for monophyly unless historical bottlenecks have accelerated coalescence. For instance, Kingston *et al.* (2009) used anonymous nuclear and mtDNA markers to elucidate the phylogenetic relationships among the Delphininae. In their analysis, *L. hosei* showed high affinity to *T. aduncus* for both marker systems, suggesting recent shared ancestry between *Tursiops* and *Lagenodelphis*. A third explanation for the occurrence of Fraser's dolphin haplotypes among the PTF-associated bottlenose dolphins is that introgression events have taken place, in which Fraser's dolphin mtDNA entered the population through hybridization.

Coastal, Indo-Pacific bottlenose dolphins of northwestern Australia

Indo-Pacific bottlenose dolphins (*T. aduncus*) inhabit near-shore areas of much of the Australian coastline (Ross 2006; Woinarski *et al.* 2014; this study). Occurring in the shallow, coastal waters of the western Pacific and Indian Oceans, Indo-Pacific bottlenose dolphins thereby occupy a niche otherwise filled by coastal ecotypes of common bottlenose dolphins in various other regions (e.g. the coastlines of New Zealand, the central and eastern Pacific Ocean, the western and eastern Atlantic Oceans and the Mediterranean Sea Natoli *et al.* 2005; Tezanos-Pinto *et al.* 2009; Moura *et al.* 2013; Fruet *et al.* 2014).

Our study also revealed a strong isolation-by-distance pattern among coastal Indo-Pacific bottlenose dolphin (sub-) populations across northwestern Australia. Fine-scale genetic structuring over scales of just tens to hundreds of kilometres should be viewed as the rule rather than the exception in coastal Australian Indo-Pacific bottlenose dolphins (e.g. Wiszniewski *et al.* 2009; Ansmann *et al.* 2012; Kopps *et al.* 2014), as it should be in coastal common bottlenose dolphins globally (Fernández *et al.* 2011; Mirimin *et al.* 2011; Moura *et al.* 2013; Browning *et al.* 2014; Fruet *et al.* 2014; Louis *et al.* 2014). Here, however, we document the existence of a genetic cline among coastal locations over some hundreds of kilometres (Beagle Bay to Coral Bay, Fig. 1).

An exception to this was the marked genetic differentiation between the dolphins sampled at the two extreme northeast coastal sites (Cygnet Bay and Beagle Bay), located in close proximity to each other (<150 km apart). The dolphins from Beagle Bay, however, clustered closely with the rest of the coastal populations, distinct from Cygnet Bay. Similar differentiation was detected between Australian snubfin dolphin populations of Cygnet Bay and Roebuck Bay (to the south of Beagle Bay), *c.* 300 km apart (Brown *et al.* 2014). The reasons for this differentiation, which was detected at a smaller spatial scale than elsewhere in the study area, are unknown. The relatively narrow, deep-water entrance to Cygnet Bay, subject to immense tidal movements (*c.* 12 m on spring tides), may act as a natural barrier to dispersal. Additional sample collection to the east of Cygnet Bay, the incorporation of detailed habitat data (e.g. bathymetry, substrate type) and large-scale genomic data will better elucidate the patterns and potential drivers of genetic connectedness among

coastal populations of bottlenose dolphins across northwestern Australia.

Conclusions and recommendations

This study provides evidence that the common bottlenose dolphin (*T. truncatus*) is the predominant species associating with the Pilbara Trawl Fishery and that haplotype sharing or recruitment from adjacent, coastal populations (*T. aduncus*) does not occur. There appears to be no genetic substructuring within the PTF-associated population. Data on population size need to be acquired before the viability, or capacity to absorb and recover from, the anthropogenic impact of ongoing incidental catch, at an estimated minimum of *c*. 50 dolphins *per annum*, can be assessed (Allen *et al.* 2014). A more complete biopsy sample data set, from offshore *T. truncatus* populations adjacent to the PTF, needs to be accumulated to allow the quantification of the levels of gene flow with adjacent, pelagic populations. This might also allow: the detection of any changes in population size due to fishery-caused mortalities (c.f., Garza & Williamson 2001); the determination of whether closely related individuals are subject to incidental capture (c.f., Mendez *et al.* 2010), which can exacerbate the demographic impacts of bycatch in highly social species, such as delphinids (Wade *et al.* 2012); and, the definition of appropriate management units for pelagic dolphins across northern Australia (c.f., Bilgmann *et al.* 2014). Finally, underwater video footage collected inside trawl nets (Jaiteh *et al.* 2014), as well as photo-identification data from around trawlers (Allen 2015), suggests that a community of dolphins within the broader population may show fidelity to foraging around trawlers. Estimating the number of individuals interacting with the trawlers is also required to better assess the level of impact this putative community faces.

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Data accessibility

Data for this manuscript have been made available on DRYAD: doi:10.5061/dryad.908g4.

Supporting information

Additional supporting information may be found in the online ver sion of this article.

Table S1 Sample vouchers from Genbank, species and corre sponding references used to provide comparison with the sam ples collected here.

Table S2 Genetic diversity indices for sampling sites for all 19 microsatellite loci.

Data S1 Recoded microsatellite data and input file for ImA analysis.

Data S2 Input file for migrate analysis.

Data S3 Input file for mtDNA analysis.

Data S4 IBD analysis.