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RESEARCH ARTICLE

Environmental and social influences on the genetic structure of bottlenose dolphins (*Tursiops aduncus*) in Southeastern Australia

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Abstract Determining genetic connectivity of bottlenose dolphin communities helps identify evolutionary mechanisms, such as environmental and social factors, that interact to shape dispersal in highly social marine mammals. Here, we expand on a localized study that found marked genetic differentiation among resident dolphins (*Tursiops aduncus*) in the Port Stephens embayment and adjacent coastal communities, to include four additional communities inhabiting different environment types along the New South Wales coast, Southeastern Australia. Analysis of the mitochondrial DNA control region and seven microsatellite loci suggest the nine communities may have originated from a single ancestral population that progressively colonised the coast in a southward direction. Gene flow among communities was predominately governed by habitat type. The two enclosed embayments showed the highest level of genetic differentiation from other communities, while genetic differentiation among coastal and open embayment communities generally followed a pattern of isolation by distance. Directional bias in recent migration rates was evident, with the centrally located Hunter coast communities consisting of individuals with mixed ancestry from the Northern, Southern and Port Stephens communities. Emigration from Port Stephens was substantially higher than in the opposite direction, indicating there may be social barriers to dispersal created by Port Stephens dolphins. Our results suggest that the scale of connectivity of bottlenose dolphin communities inhabiting heterogeneous environments is likely to be affected by local habitat adaptation. This has important implications for the management of communities exposed to increasing levels of anthropogenic disturbances, such as the intensive commercial dolphin-watching industry operating in Port Stephens.

Keywords Population genetics · Phylogeography · Habitat · Conservation management

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Introduction

Identifying genetic discontinuities in a species' range is critical when evaluating the evolutionary processes affecting the distribution of genetic variation within and amongst populations. The level of genetic structure is often governed by both demographic and environmental factors, including life-history characteristics, dispersal patterns, population size, social organisation, habitat connectivity and resource distribution (Chesser 1991; Surridge et al. 1999; Beheregaray and Sunnucks 2001; Dobson et al. 2004; Fraser et al. 2004; Archie et al. 2008; Worthington Wilmer et al. 2008). Detecting biologically meaningful

units has also become a heightened conservation priority for an increasing number of species as a result of anthropogenic disturbances to both terrestrial and marine ecosystems. Maintaining levels of genetic diversity may help preserve a population's evolutionary potential (Frankham et al. 2003), while understanding the genetic and ecological relationships among populations provides critical information for the design and implementation of effective management initiatives (Palumbi 2004; Palsbøll et al. 2007).

Cetacean species show great variation in spatial genetic structure (e.g., Hoelzel 1998; Lyrholm et al. 1999). This variation has been attributed to the complex interaction between historical factors, such as colonisations and changes in the marine environment, and contemporary factors, such as the utilisation of different habitats, resource specialisations, social structure and aspects of life history and demography (Hoelzel 1998; Rosel et al. 1999; Natoli et al. 2004; Hoelzel et al. 2007; Möller et al. 2007). The geographic scale at which bottlenose dolphins (*Tursiops* spp.) show genetic structure appears highly dependent on the type of environment the population inhabits. Little differentiation has been observed with both nuclear and mitochondrial DNA markers in large pelagic populations (e.g., Quérouil et al. 2007), while dispersal of coastal dolphins is often restricted, especially in resident embayment populations (Krützen et al. 2004; Sellas et al. 2005; Parsons et al. 2006; Möller et al. 2007). Genetic discontinuities in some bottlenose dolphin populations coincide with breaks in oceanographic features, such as water depth, bottom topography, primary productivity, sea surface temperature and salinity. For example, in South Australia, Bilgmann et al. (2007a) attributed high genetic differentiation between dolphins in the Spencer Gulf and the adjacent coastal population to the strong temperature and salinity differential caused by the oceanographic front at the mouth of the gulf. Similar correlations have been documented in bottlenose dolphins from the Black Sea to Scotland (Natoli et al. 2005) and between coastal and offshore ecotypes in the North-West Atlantic (Hoelzel et al. 1998).

For bottlenose dolphins inhabiting coastal environments, high site fidelity resulting from local adaptation to different ecological conditions, combined with differential resource use, are potential evolutionary mechanisms promoting fine-scale genetic structure (Hoelzel 1998). In New South Wales (NSW) in particular, the genetic divergence of a resident *T. aduncus* population in the Port Stephens embayment from nearby communities on the adjacent coastline has occurred despite intermittent interactions and ample opportunities for mating between these dolphin communities (Möller et al. 2007). Similarly, significant differentiation was detected between embayment and coastal *Tursiops truncatus* communities on a comparable spatial scale in Florida (Sellas et al. 2005). Parallels in habitat characteristics between these regions (i.e., enclosed embayments characterised by extensive seagrass beds and mangroves, and coastal habitats consisting of sandy beaches and rocky reefs) suggest that intraspecific variability in habitat selection may be a strong evolutionary force acting on population structure in bottlenose dolphins. This hypothesis is supported by fine-scale differences in fish assemblages between distinct habitats (e.g., Gray et al. 1996), as well as differences in diets (e.g., Gannon and Waples 2004) and foraging specialisations (e.g., Sargeant et al. 2007) among bottlenose dolphins. Further, a recent study on the social network structure of dolphins in Port Stephens showed that social and spatial segregation of communities coincided directly with a change in habitat type (estuarine vs. marine, Wiszniewski et al. 2009). In addition, several studies on other highly mobile marine and terrestrial species have shown that rates of dispersal can be strongly affected by environmental conditions (e.g., Sacks et al. 2004; Watts and Johnson 2004). For bottlenose dolphins, efficient prey exploitation using specialised techniques may involve a significant social learning component (e.g., Krützen et al. 2005), thus dispersal from a natal habitat may reduce an individual's fitness. This applies particularly to female bottlenose dolphins, as their fitness is highly dependent on familiarity with resources in order to provide for calves, as well as familiarity with conspecifics for the protection of calves (Connor et al. 2000). As a consequence, reduced levels of gene flow among inshore communities typically results from high philopatry and long-term social affiliations between females, as well as moderate levels of male philopatry (Connor et al. 2000; Duffield and Wells 2002; Krützen et al. 2004; Möller and Beheregaray 2004; Möller et al. 2006).

Identification of discrete genetic units is especially important for the conservation and management of coastal cetaceans as a consequence of escalating levels of human activities in coastal areas. Changes in habitat use, reduced reproductive levels and/or higher mortality rates in bottlenose dolphins have been linked to entanglements in shark nets (Cockcroft 1990) and interactions with commercial and recreational fishing operations (Shaughnessy et al. 2003), aquaculture (Watson-Capps and Mann 2005), recreational boating traffic (Allen and Read 2000) and dolphin-based tourism (Bejder et al. 2006; Lusseau et al. 2006). Port Stephens, NSW, is the self-proclaimed dolphin watching capital of Australia, where a multi-vessel commercial industry targets a resident dolphin community (Allen et al. 2007). This is of particular concern as short-term changes in dolphin behaviour and group stability as a result of tour boat approach have been observed (Allen 2005). While documented tourism impacts and the genetic

distinctiveness of the *T. aduncus* population may provide grounds to apply regulatory measures to tour operators in this newly designated Marine Protected Area, limited empirical data exists on other sources of anthropogenic disturbance to dolphins and the ecosystems they inhabit in NSW as a whole.

Several localised *T. aduncus* communities are sighted year-round along the NSW coast, residing in shallow embayments and estuaries, and in coastal waters generally within a few kilometres from shore. At the central latitude of NSW, the Port Stephens dolphins represent a distinct population from communities found on the adjacent Hunter coast (Möller et al. 2007). However, the scale at which the adjacent Hunter coastal population extends, in addition to the number of genetically distinct populations in NSW, is unknown. In light of this uncertainty, this study uses a combination of microsatellite and mtDNA markers to assess population genetic structure and levels of gene flow among nine inshore *T. aduncus* communities inhabiting different environments along the entire NSW coast. Based on the initial findings in Möller et al. (2007), we have two contrasting general predictions that we aim to test for the NSW coastal region. First, embayment and adjacent coastal dolphin communities should display restricted levels of dispersal, despite their geographic proximity. Secondly, due to the general absence of historical biogeographic barriers along the NSW coast (e.g., Waters et al. 2005; Banks et al. 2007), we predict that coastal communities should show a pattern of isolation by distance. Through the identification of genetically discrete populations, we aim to further our understanding of the complex interaction between environmental and social influences that may increase levels of genetic structure between coastal bottlenose dolphin communities. Further, with the recent designation of several Marine Protected Areas throughout NSW coastal waters, information on population boundaries will help determine the necessary spatial and temporal scale for conservation and management efforts needed to preserve levels of genetic diversity in this species.

Methods

Study sites and sample collection

Bottlenose dolphin skin samples were collected between 1999 and 2007 from nine communities distributed along the 1,060 km NSW coastline (Fig. 1). These included samples from the Port Stephens (PS) and Hunter coastal communities analysed in Möller et al. (2007): Eastern PS ($N = 20$), Western PS: ($N = 15$), Newcastle ($N = 20$), Broughton Island ($N = 21$) and Forster ($N = 10$). An additional 24 samples from Port Stephens and 80 samples from four other communities in Southern and Northern NSW were added to this study. These four new sampling localities (from South to North) included: Eden ($N = 22$), a community inhabiting an open embayment and open coastline of far Southern NSW; Jervis Bay (JB; $N = 23$), a community consisting of around 80 residents in a large open embayment (Möller and Harcourt 1998); Yamba ($N = 21$), a protected estuary inhabited by about 70 individuals (Fury and Harrison 2008); and Ballina ($N = 14$), a large community that regularly interacts with dolphins further north around Byron Bay (Hawkins 2008). Skin samples were obtained using either a PAXARMS (Timaru, New Zealand) biopsy rifle (Krützen et al. 2002) or a biopsy pole (Bilgmann et al. 2007b). Samples were preserved in 20% dimethyl sulphoxide (DMSO) saturated with sodium chloride (Amos and Hoelzel 1991) or 100% ethanol. In Port Stephens and Jervis Bay, samples used for analysis were restricted to animals considered resident from long-term photo-identification studies (Möller et al. 2002). Furthermore, samples from dependent calves were not included in the analysis from any sampling site.

Genetic methods

DNA was extracted from biopsy samples using a salting-out protocol (Sunnucks and Hales 1996). Individuals were sexed by the amplification of fragments from the SRY and ZFX genes using polymerase chain reaction (PCR; Gilson et al. 1998). All samples analysed in this study were genotyped at seven cetacean microsatellite loci (EV1 and EV37 (Valsecchi and Amos 1996); MK5, MK6 and MK8 (Krützen et al. 2001); KW2 and KW12 (Hoelzel et al. 1998)) in 10 µl radio-labelled reactions as described in Möller and Beheregaray (2004). PCR products were separated using 6% polyacrylamide gel electrophoresis and visualised by autoradiography. To assure accuracy in genotyping and to standardise allele sizing for each locus, several control samples from the previous study were re-amplified for each new gel and all samples were independently scored by at least two people. A 460 bp fragment of the mtDNA control region was amplified by PCR with the primers Dlp-1.5 (5′-TCACCCAAAGCTGRARTTCTA-3′) and Dlp-5 (5′-CC ATCGWGATGTCTTATTTAAGRG GAA-3; Baker et al. 1993) in a 10 µl radio-labelled reaction (conditions specified in Möller and Beheregaray 2001). PCR products were run on a non-denaturing polyacrylamide gel to screen amplified fragments for sequence variation by the single-stranded conformation polymorphism (SSCP) method as described by Sunnucks et al. (2000). Fresh PCR products were purified with Ultraclean™ 15 DNA Purification Kit (Gels and Solutions) for all unique SSCP phenotypes, and at least one additional individual representing each phenotype to confirm that individuals with the same SSCP phenotype

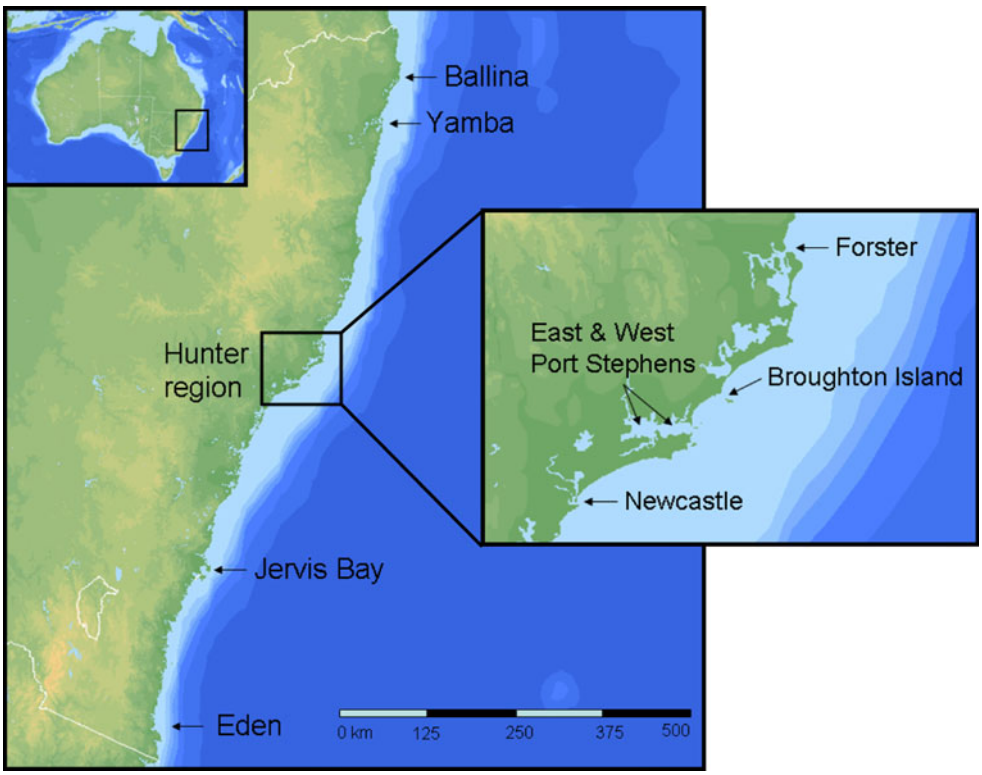

Fig. 1 Map of nine sampling localities of bottlenose dolphins along the New South Wales coast (NSW), Southeastern Australia

had identical sequences. Products were sequenced in an ABI 3700 Automated DNA sequencer (Perkin Elmer) following manufacturer's instructions. MtDNA sequences were cleaned and aligned using SEQUENCER 3.0 (Gene Codes Corporation, Ann Arbor, MI) resulting in a 403 bp fragment.

Data analysis

Genetic variation

Microsatellite genotypes were screened for duplicate sampling using MStools 3.1 (Park 2001) and tested for genotypic errors using the software MICRO-CHECKER (Van Oosterhout et al. 2004). After removal of duplicates, genetic variation within each sample area was estimated by calculating allelic richness, number of unique alleles and inbreeding coefficients (F_{IS}) using the program FSTAT 2.9.3 (Goudet 2001) and observed and expected heterozygosity using ARLEQUIN 3.01 (Excoffier et al. 2005). Tests for significant deviations from Hardy–Weinberg equilibrium (HWE) at each community were conducted using Fisher's exact test and the Markov chain method with 1,000 iterations, within GENEPOP 3.4 (Raymond and Rousset 1995). Significance values for multiple comparisons were adjusted by Bonferroni correction (Rice 1989). Previous tests for linkage disequilibrium demonstrated independent assortment of the seven loci used in this study (Möller et al. 2001). In addition, the index of relatedness developed by Queller and Goodnight (1989) was used to estimate average pairwise relatedness for each community and test if any community had a higher number of related individuals than expected under the null hypothesis of a randomly mating population. The computed distributions were compared to a distribution of 1,000 simulated individuals generated using each community's allele frequencies in the software KINSHIP 1.2 (Goodnight and Queller 1998) and significance was assessed using a Kolmogorov–Smirnov two-sample test.

Haplotypic (h) and nucleotide (π) diversity for mtDNA data was calculated for each population using ARLEQUIN. The Kimura 2-parameter (K2P) genetic distance (Kimura 1980) was used with a gamma distribution of 0.5 to allow for unequal substitution rates among sites. A haplotype network was constructed to examine the genealogical relationships among mtDNA control region lineages using the parsimony method of Templeton et al. (1992). The maximum number of substitutions to parsimoniously connect two haplotypes with 95% confidence was estimated by firstly linking sequences with the smaller number of differences. In addition, this method estimates haplotype outgroup probabilities, which allows the most ancient haplotype in the sample to be identified. The analysis was performed within the program TCS, v 1.06 (Clement et al. 2000).

Genetic differentiation

For both microsatellite and mtDNA data, levels of genetic differentiation between pairs of localities were investigated by computing F_{ST} (Weir and Cockerham 1984). Significance for all pairwise comparisons was assessed with 10,000 permutations using ARLEQUIN. Statistical power of both datasets to detect genetic heterogeneity at various levels of true differentiation (defined as F_{ST}) among communities was estimated using the program POWSIM (Ryman and Palm 2006). We used present sample sizes, number of loci and allele frequencies to simulate the segregation of a base population into 1,000 random sets of nine populations to predefined levels of divergence. For mtDNA analysis, the number of samples was halved to represent the number of haploid genes present in each community (Larsson et al. 2008). The program then tests for genetic homogeneity at each locus using the Fisher's exact test, with the proportion of significant results ($P < 0.05$) combined over all loci through the Fisher's method indicating the power of the data (Ryman and Palm 2006; Ryman et al. 2006). To test for any relationship between geographical distance and genetic distance, we used Mantel permutation tests (Mantel 1967) and spatial autocorrelation analysis (Smouse and Paetkau 1999). Geographical distances were calculated using the shortest distance via the coastal marine environment. Mantel tests were performed between matrices of F_{ST} and geographical distance using FSTAT 2.9.3 (Goudet 2001). Analyses were conducted using all data followed by a subset of the data excluding Port Stephens and Yamba (the two enclosed embayment communities) and statistical significance was determined through 10,000 iterations. Spatial genetic structure at the individual level was assessed using a multivariate spatial autocorrelation approach developed for codominant loci, within the program GenAlEx 6 (Smouse and Paetkau 1999; Peakall and Smouse 2006). An autocorrelation coefficient r was plotted between individuals from the same community (where the distance class equals 0) and between individuals separated by 16 variable distance classes (covering the geographic separation of all communities) to assess the genetic similarity between pairs of individuals falling within each distance class. 95% confidence intervals (CI) about the null hypothesis of no spatial genetic structure were determined from 1,000 permutations, and the 95% CI of r estimates were calculated by 1,000 bootstrap pairwise comparisons. Statistical significance was declared when the 95% CI of r fell outside the CI about the null hypothesis of $r = 0$ (Peakall et al. 2003).

To estimate the number of genetically distinct populations along the NSW coast, a Bayesian model-based clustering method as implemented in STRUCTURE 2.1 (Pritchard et al. 2000), was used. Assuming Hardy–Weinberg and linkage equilibrium, STRUCTURE probabilistically determines the most likely number of clusters (K) by calculating the log likelihood value of the data, while assigning each multilocus genotype to a genetic cluster. Since the two communities in Port Stephens and three in the Hunter coast region have previously been assigned to two distinct populations (Möller et al. 2007), Markov chain Monte Carlo (MCMC) runs were conducted for K values ranging from one to six using a burn-in period of 100,000 iterations followed by runs of 10^6. Five independent runs were conducted for each value of K to check for convergence. The analysis was first performed without population information, followed by runs with population membership defined a priori. Given the close geographical proximity of communities and presumably moderate levels of gene flow, the admixture model was chosen using the correlated frequency model (Falush et al. 2003). The number of populations (K) most compatible with the observed data was obtained by maximising the estimated mean log-likelihood of the data for different values of K (Pritchard et al. 2000).

Contemporary migration rates (m) were estimated for NSW bottlenose dolphins in BAYESASS, which implements a Bayesian procedure using MCMC techniques (Wilson and Rannala 2003). This approach can provide relatively accurate estimates of asymmetric migration rates in populations deviating from Hardy–Weinberg and migration-drift equilibrium if the level of genetic differentiation between populations is moderate ($F_{ST} \geq 0.05$), and assumptions of linkage equilibrium, small and constant migration rates, and stable allele frequencies over recent generations, are met (Faubet et al. 2007). To improve precision, communities were pooled according to F_{ST} results into four geographic regions: Northern, Hunter coast, Port Stephens and Southern NSW. This ensured that sample sizes were relatively large and equal between regions, thereby facilitating the accuracy of estimates. MCMC runs of 5.0×10^6 iterations were conducted and sampled every 2,000 iterations to infer posterior probability distributions of parameters. The initial burn-in period of 10^6 was discarded to ensure model parameters were sufficiently randomised. The default delta values of 0.15 were used since the acceptance rates varied between the recommended 40–60% of total iterations (Faubet et al. 2007). To examine the strength of the information in the data, 95% confidence intervals were determined for the migration rate and compared to those that occur when there is insufficient data to affect the posterior distribution of the migration rate.

Sex-biased dispersal

To test for a bias in dispersal in NSW bottlenose dolphins, sex-specific comparisons were performed using mean

corrected assignment indices (AIc) and mean relatedness for six distinct regions based on F_{ST} results and spatial autocorrelation analysis: Eden, Jervis Bay, Port Stephens, Hunter coast, Yamba and Ballina. Firstly, the partially Bayesian classification method (Rannala and Mountain 1997) implemented in GENECLASS 2 (Piry et al. 2004) was used to calculate male and female AIc values for each region and sex-based differences were tested using the Mann–Whitney *U*-test in SPSS 15.0. Secondly, mean relatedness (*R*) was calculated within (MM; FF) and between (MF) sexes in each region with standard errors obtained by jackknifing over all loci (Queller and Goodnight 1989). This was conducted using the software RELATEDNESS 5.04 (Goodnight and Queller 1998) and differences in the mean relatedness among categories was assessed using a two-sample randomisation test with 10,000 iterations in the program RT 2.1 (Manly 1997).

Results

Genetic variation

After five duplicate samples were removed from the dataset based on identical genotypes at seven microsatellite loci, the same mtDNA haplotype and matching sex, 185 samples from nine communities were available for genetic analyses (Table 1). No evidence for null alleles or other genotypic errors was observed for the seven microsatellite loci examined. Following sequential Bonferroni corrections, there was no evidence for deviation from HWE for all communities across all loci. Measures of nuclear genetic variation including allelic richness, heterozygosity and number of unique alleles, was generally highest in the northern communities and lowest in the two Port Stephens communities (Table 1; Appendix). There was also no support for inbreeding at any locality based on F_{IS} estimates, while average relatedness estimates for each community were low, ranging from −0.025 to −0.135 (Table 1).

Sequence alignment of the 403 bp fragment of the mtDNA control region from nine communities revealed ten polymorphic sites, defining eight unique haplotypes (GenBank accession numbers AF287951-3, EF581128, GQ420670). All haplotypes were closely related (Fig. 2), with two to five haplotypes found in each community (Table 2). As a result, low haplotypic (*h*) and nucleotide (π) diversity was observed (*h*: 0.247–0.649; π: 0.0018–0.0063), especially for Eden, West Port Stephens and Yamba communities (Table 2). While the highest frequency haplotype, SEAust 2, was continuously distributed throughout NSW, SEAust 8 was identified as the ancestral maternal lineage and was restricted to the two northernmost communities.

Genetic differentiation

Highly significant population structure based on F_{ST} with microsatellite data was detected for most pairwise comparisons of NSW communities (Table 3). Yamba and Port Stephens communities displayed highest levels of differentiation to all other sampling localities ($F_{ST} = 0.039$–0.182, $P < 0.01$), while gene flow also appears limited between Southern (Eden and Jervis Bay) and Northern (Hunter coast and Ballina) communities. There was low but significant structure between East and West Port Stephens communities ($F_{ST} = 0.0187$, $P < 0.05$), which was not detected in the previous study by Möller et al. (2007). Although a larger East Port Stephens sample size used here may include a higher proportion of relatives producing a significant result, this is unlikely since F_{IS} was negative for both communities and neither one contained a significantly higher number of related individuals than expected from a randomly mating population (Table 1). POWSIM analysis suggested the microsatellite data set contained sufficient statistical power to detect accurate levels of differentiation despite smaller sample sizes for a few communities. For instance, the probability of detecting true genetic differentiation of $F_{ST} = 0.01$ (representing all but one pairwise comparison) was 99% and increased to 100% for F_{ST} values above 0.02. The α (type 1) statistical error, which represents the probability of obtaining false significance when there is no differentiation, was low (6.5%). Therefore F_{ST} estimates support the suggestion of six distinct populations: Eden, Jervis Bay, Port Stephens, the Hunter coastal region, Yamba and Ballina (in addition to the genetic division of two Port Stephens communities).

Tests for associations between genetic divergence (F_{ST}) and geographic distance revealed no significant correlation when all nine communities were analysed ($r = 0.251$, $P = 0.14$). This resulted from highly significant differentiation between Port Stephens and the Hunter coastal communities over distances as small as 23 km and considerably lower F_{ST} values among sampling sites along the coast separated by up to 640 km. F_{ST} values were also higher for Yamba comparisons relative to its geographic separation to other communities. Exclusion of Port Stephens (East and West) and Yamba dolphins, which reside in relatively enclosed embayments, produced a significant correlation for the remaining six communities ($r = 0.804$, $P = 0.0004$), indicating that geographic distance plays a role in the distribution of genetic variation among bottlenose dolphins along the coast. The results from the spatial autocorrelation analysis in GenAlEx mirrors the isolation by distance analysis conducted for all nine communities. Individuals from the same community (0 km distance class) and those separated by 20 km were significantly more similar than expected from random ($P = 0.001$; Fig. 3),

Table 1 Summary of genetic variability in NSW bottlenose dolphins based on seven microsatellite loci

Population (abbrev)	*N* (*m*:*f*)	UA	AR	H_o	F_{IS} (*P* value)	$R \pm$ SE (*P* value)
Eden (ED)	22 (13:9)	3	3.95	0.63	0.150 (0.380)	−0.048 ± 0.0004 (0.290)
Jervis Bay (JB)	22 (14:8)	1	4.02	0.65	0.045 (0.179)	−0.048 ± 0.0007 (0.03)
West PS (WPS)	17 (9:8)	0	3.31	0.59	−0.034 (0.185)	−0.065 ± 0.0016 (0.378)
East PS (EPS)	42 (20:22)	1	3.52	0.58	−0.077 (0.102)	−0.025 ± 0.0001 (0.292)
Newcastle (NC)	19 (8:11)	1	4.13	0.59	0.053 (0.114)	−0.058 ± 0.0006 (0.186)
Broughton Island (BI)	20 (13:7)	3	4.16	0.70	−0.020 (0.369)	−0.055 ± 0.0013 (0.354)
Forster (FOR)	9 (2:7)	0	3.56	0.63	0.012 (0.433)	−0.135 ± 0.0069 (0.291)
Yamba (YAM)	20 (12:8)	9	4.38	0.65	0.029 (0.258)	−0.056 ± 0.0020 (0.010)
Ballina (BB)	14 (4:10)	4	4.30	0.74	−0.042 (0.241)	−0.078 ± 0.0010 (0.345)

Abbreviated names follow each community; *N* number sampled (and of each sex), *UA* number of unique alleles, *AR* allelic richness, H_o mean observed heterozygosity, F_{IS} inbreeding coefficient, *R* average pairwise relatedness estimates, *SE* standard error

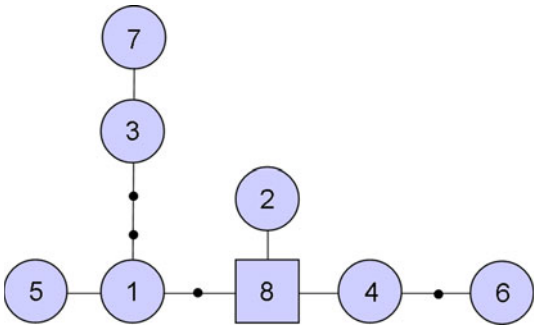

Fig. 2 Parsimony network of mtDNA control region haplotypes identified from bottlenose dolphins in NSW. Connections between haplotypes represent one base-pair differences and *filled circles* represent additional single-point mutations. Haplotype SEAust 8 was identified as the ancestral lineage based on coalescence theory and is displayed by a *square*

while for pairs of individuals separated by more than 38 km (*x*-axis intercept of the correlogram), the autocorrelation coefficient (*r*) became non-significant and oscillated considerably. This pattern of spatial autocorrelation is analogous to the 'stabilising profile' described by Diniz-Filho and Telles (2002), where there is a combination of high and low levels of genetic differentiation for samples separated by larger geographic distances.

The Bayesian clustering approach implemented in STRUCTURE was used to test whether genetic structure could be detected in the absence of geographic data and to determine the number of inshore bottlenose dolphin populations in NSW. Irrespective of the allele frequency model chosen or inclusion of sampling location information, the posterior probability [Ln *P*(*D*)] was highest at $K = 4$ (Fig. 4). However, based on individual assignment probabilities (Fig. 5), the most likely number of clusters for this data set appears to be three. For instance, at $K = 4$, the Southern (Eden and Jervis Bay) and Northern communities (Yamba and Ballina) were clearly distinct, however, all Port Stephens individuals had symmetric assignments to two populations (Fig. 5b). At $K = 3$, most individuals where strongly assigned to one of three clusters (Fig. 5a), which is an indicator of real population structure (Pritchard and Wen 2004). The three clusters corresponded to populations in Southern NSW (Eden and Jervis Bay), Northern NSW (Yamba and Ballina) and Port Stephens. Individuals from the Hunter coast communities were of mixed ancestry,

Table 2 Percentage frequency and genetic diversity of mtDNA control region haplotypes (SEAust 1–8) for NSW bottlenose dolphins, where *NH* number of haplotypes

	1	2	3	4	5	6	7	8	NH	Haplotypic diversity	Nucleotide diversity
ED	14	86							2	0.247 (0.108)	0.0018 (0.0016)
JB	40	45	5	5		5			5	0.649 (0.065)	0.0052 (0.0034)
WPS	18	82							2	0.309 (0.122)	0.0023 (0.0019)
EPS	43	50	5		2				4	0.577 (0.039)	0.0044 (0.0029)
NC	16	68	11				5		4	0.521 (0.123)	0.0046 (0.0031)
BI	40	55	5						3	0.563 (0.063)	0.0043 (0.0029)
FOR	11	33	56						3	0.639 (0.126)	0.0058 (0.0040)
YAM		80	10					10	3	0.358 (0.127)	0.0018 (0.0016)
BB		14	57					29	3	0.615 (0.102)	0.0063 (0.0041)

Table 3 Genetic differentiation among nine bottlenose dolphin communities in NSW

	ED	JB	WPS	EPS	NC	BI	FOR	YAM	BB
ED		0.167*	−0.049	0.184**	0.043	0.149*	0.481***	−0.040	0.401***
JB	0.026**		0.108	−0.025	0.047	−0.037	0.221*	0.167**	0.181*
WPS	0.137***	0.146***		0.130*	0.009	0.087	0.415**	−0.041	0.341**
EPS	0.092***	0.114***	0.019*		0.059	−0.033	0.233*	0.190**	0.198**
NC	0.025**	0.041***	0.099***	0.044***		0.015	0.168	0.062	0.140*
BI	0.037***	0.041***	0.082***	0.045***	0.011		0.218*	0.157*	0.178*
FOR	0.029*	0.025	0.126***	0.060**	0.019	−0.003		0.497**	−0.078
YAM	0.085***	0.060***	0.182***	0.154***	0.101***	0.057***	0.039*		0.409***
BB	0.082***	0.072***	0.146***	0.130***	0.078***	0.037***	0.015	0.044**	

Pairwise F_{ST} values calculated with microsatellite data (matrix below) and from the 403-bp fragment of the mtDNA control region (above matrix)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

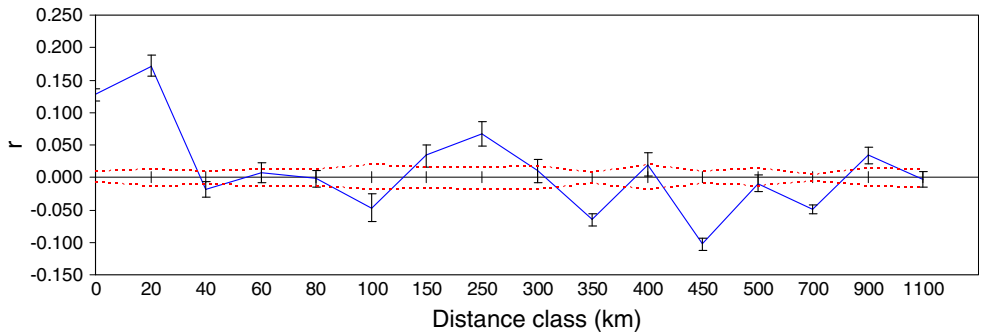

Fig. 3 Correlogram displaying the spatial genetic autocorrelation (r) as a function of variable distance classes that span the full geographic distribution of sampled localities in NSW. 95% CI about the null hypothesis of no genetic structure is illustrated by the *dotted lines* and *error bars* about r indicate 95% CI as determined by bootstrapping

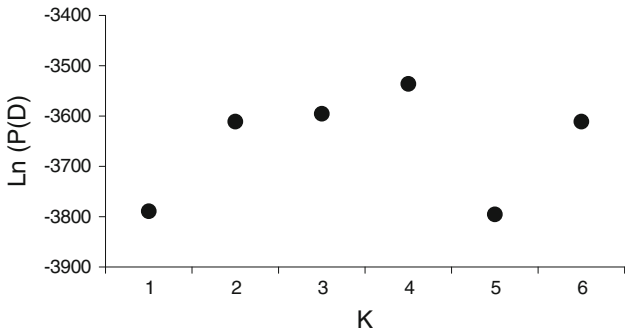

Fig. 4 Changes in the estimated posterior probabilities averaged over five replicate runs for values of $K = 1$–6 using the Bayesian method in STRUCTURE

indicating higher levels of gene flow into this region from the three populations detected. Surprisingly, based on F_{ST} results, STRUCTURE grouped Yamba and Ballina despite significant differentiation of these communities ($F_{ST} = 0.044$, $P < 0.01$). These results were confirmed by subsequent runs excluding all other communities, and may have resulted from the smaller Ballina sample size.

An additional Bayesian method in BAYESASS was used to estimate recent migration rates of bottlenose dolphins from four NSW regions: Northern, Hunter coast, Port Stephens and Southern NSW. Simulations where no information was contained in the data to estimate migration rates produced 95% CI of 0.675–0.992 for the proportions of non-migrant individuals in each region and 95% CI of 0.000155–0.218 for migrants. Confidence intervals obtained from the data set were considerably smaller than those obtained randomly (Table 4), suggesting that sufficient information was available to reliably estimate migration rates. Corroborating findings from STRUCTURE, the Southern, Northern and Port Stephens regions had a high proportion of individuals identified as non-migrant (98–99%) while the Hunter coastal region was admixed with only a 68% proportion of non-migrants (Table 4; Fig. 6). Migration rate estimates were on average very low, with slightly higher rates in the direction of the Hunter coast region from all other areas. Given its geographic proximity, Port Stephens was predictably the source of most migrants into the Hunter coast ($m = 0.26$; 95% CI: 0.181–0.309), however, migration in the opposite direction was very low ($m = 0.003$; 95% CI: 0–0.016).

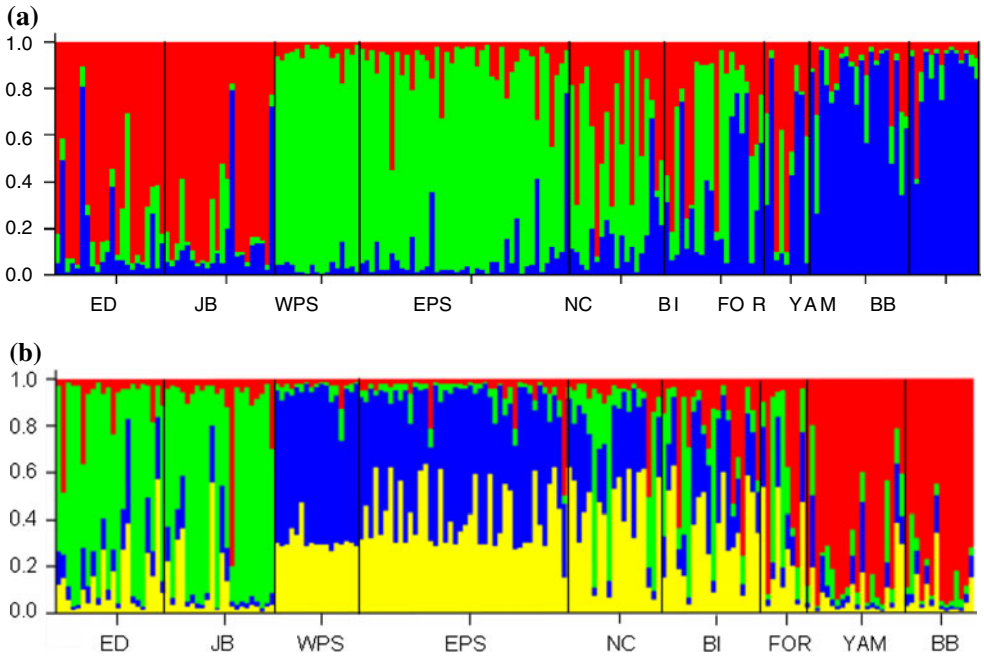

Fig. 5 Likelihood assignments based on seven microsatellite loci for **a** $K = 3$ and **b** $K = 4$. Individuals are represented by *columns with black lines* distinguishing the nine communities analysed in this study

Table 4 Mean ± SD (95% CI) posterior distributions for migration rates among bottlenose dolphins in NSW calculated with the program BAYESASS

Migration rate	To			
From	Southern NSW	Port Stephens	Hunter coast	Northern NSW
Southern NSW	**0.984 ± 0.014**	0.003 ± 0.003	0.046 ± 0.033	0.007 ± 0.010
$N = 44$	**(0.950–0.999)**	(0–0.013)	(0.003–0.128)	(0–0.035)
Port Stephens	0.005 ± 0.008	**0.992 ± 0.007**	0.259 ± 0.033	0.008 ± 0.137
$N = 59$	(0–0.026)	**(0.971–0.999)**	(0.181–0.309)	(0–0.053)
Hunter coast	0.005 ± 0.007	0.003 ± 0.004	**0.680 ± 0.013**	0.005 ± 0.008
$N = 48$	(0–0.024)	(0–0.016)	**(0.667–0.712)**	(0–0.026)
Northern NSW	0.006 ± 0.008	0.002 ± 0.004	0.015 ± 0.015	**0.980 ± 0.021**
$N = 38$	(0–0.029)	(0–0.013)	(0–0.053)	**(0.919–0.999)**

Values along the diagonal (bold) are the proportion of individuals each generation that are not migrants. 95% CI that occur when there is no information in the data are 0.675–0.992 for the proportions of individuals derived from the source populations each generation and 0.000155–0.218 for migration rates

Patterns of genetic structure from the maternally inherited mtDNA marker were not as evident as those observed with microsatellite data (Table 3). Northern (Ballina, Yamba) and Southern (Eden, Jervis Bay) communities showed considerable levels of differentiation with most other communities, however, non-significant differentiation between several pairs of geographically distant communities also occurred. This likely resulted from the high frequency of SEAust 2 haplotype in Eden, Port Stephens and Yamba. On the other hand, highly significant F_{ST} values observed for Forster and Ballina comparisons were likely due to the dominant SEAust 3 haplotype. A power analysis of the mtDNA dataset estimated power at 0.8 for $F_{ST} = 0.05$ and 0.98 when $F_{ST} = 0.1$. In the absence of clear lineage sorting and the close genealogical relationships among all haplotypes, we can deduct that NSW communities are closely related from a historical perspective.

Sex-biased dispersal

Females in Port Stephens were found to be more philopatric than males, as estimated by significantly higher assignment values ($P < 0.05$) and mean relatedness

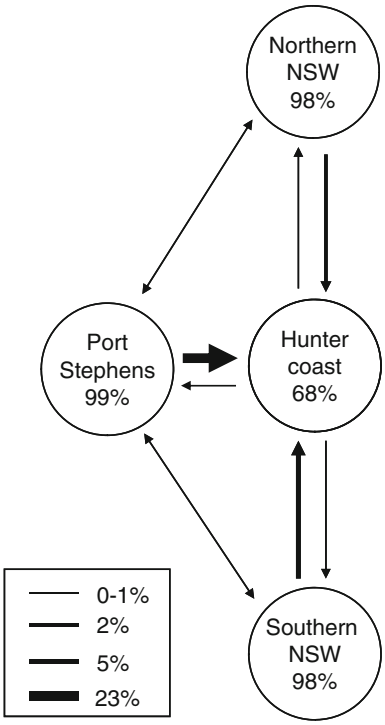

Fig. 6 Schematic diagram of recent migration rates among the four geographic regions in NSW. The percentage of individuals each generation that are non-migrants are shown inside the *circles*, while the proportion of migrants to each region are represented by the *arrow line width*

($P < 0.01$) among females than among male dolphins (Table 5). In Jervis Bay, relatedness among females was also higher than male–male and male–female comparisons ($P < 0.05$), although differences in AIc values were not significant for this community. Conversely, no significant differences were observed for the coastal communities in Eden and Ballina or the Yamba embayment community ($P > 0.05$). The Hunter coast communities showed a different dispersal pattern where mean relatedness was significantly lower among females than among males ($P < 0.01$). The probability of females being born locally was also slightly lower than that of males although the difference was not statistically significant (Table 5).

Discussion

Spatial scale of population structure

This study revealed considerable levels of genetic differentiation between most resident bottlenose dolphin communities along the NSW coast using nuclear DNA markers. Taken together with the close genealogical relationships among mtDNA control region haplotypes, the observed levels of population structure are likely a consequence of founder events originating from a single ancestral population, followed by recent restrictions to gene flow. NSW haplotypes are closely related to *T. aduncus* haplotypes sampled in Chinese waters in the Indo-Pacific Ocean (Wang et al. 1999), with maximum divergence of only 2.2% (Möller and Beheregaray 2001). Thus, progressive colonisation of coastal regions in Southeastern Australia is likely to have occurred from North to South, perhaps when suitable habitat became available after the last Holocene marine transgression (Roy 1984). The comparatively high levels of nuclear variation (including a higher number of private alleles) and the exclusive presence of the putative ancestral maternal lineage SEAust 8 in the Northern communities (Yamba and Ballina) are consistent with this hypothesis.

Our analyses also suggest at least three highly distinct populations: Northern NSW, Port Stephens and Southern NSW. Further subdivision of these populations is evident based on significant F_{ST} comparisons, especially between the Yamba embayment and Ballina in Northern NSW. The fact that the Bayesian clustering approach in STRUCTURE did not detect these partitions most likely results from the limitations of the program to detect genetic differentiation when F_{ST} values are low (Latch et al. 2006) or when populations are not in Hardy–Weinberg equilibrium. Concordant patterns of genetic differentiation emerging from this study and Möller et al. (2007) add to the growing body of evidence suggesting that embayments in general may contain genetically differentiated dolphin populations. For instance, three small

Table 5 Mean relatedness between same-sex and opposite pairs ± SE obtained by jacknifing over all loci. Corrected assignment indices (AIc) for male and female dolphins are also given for each NSW dolphin population

	Relatedness			AIc			
	MM	FF	MF	Males	Females	*t*	*P*
ED	−0.0187 ± 0.0349	−0.0718 ± 0.0564	−0.0655 ± 0.0242	0.03	−0.20	0.26	0.80
JB	−0.0803 ± 0.0142	0.0433 ± 0.0668	−0.0356 ± 0.0161	−0.26	0.45	−0.95	0.35
PS	−0.0602 ± 0.0256	0.0113 ± 0.0242	−0.0112 ± 0.0025	−0.49	0.44	−2.16	0.04
Hunter coast	−0.0006 ± 0.0298	−0.0484 ± 0.0258	−0.0193 ± 0.0027	0.14	−0.13	0.38	0.70
YAM	−0.0586 ± 0.0297	0.0034 ± 0.0867	−0.0664 ± 0.0184	0.10	−0.16	0.22	0.82
BB	−0.1150 ± 0.0812	−0.0480 ± 0.0276	−0.0819 ± 0.0168	0.18	−0.07	0.20	0.84

embayment communities in the Gulf of Mexico were found to be significantly differentiated from nearby coastal bottlenose dolphins (Sellas et al. 2005), while in South Australia, bottlenose dolphins sampled in the Spencer Gulf were genetically distinct from those ranging along the open coast (Bilgmann et al. 2007a). Natoli et al. (2005) further showed that the two ecologically distinct Mediterranean Sea basins also hold genetically differentiated bottlenose dolphin populations. With greater resolution in this present study than Möller et al. (2007), we similarly detected significant genetic differences between two socially and spatially structured communities within the small Port Stephens embayment. Negative F_{IS} values refuted the possibility that the non-random distribution of genetic variation resulted from communities composed of family groups. Instead, the direct correlation between spatial segregation and change in habitat characteristics (marine versus estuarine, Wiszniewski et al. 2009) indicates that high site fidelity and habitat adaptation are likely to be influencing the distribution of genetic variation within the population. Taken together, the strong correlation between genetic subdivisions and environmental discontinuities found in these and several other studies (e.g., Hoelzel et al. 1998; Nichols et al. 2007) provides strong evidence that adaptation to local environmental conditions can generate substantial barriers to gene flow in bottlenose dolphins.

Analysis of sex-specific dispersal patterns using a larger sample size than that in Möller and Beheregaray (2004) supported a pattern of male-biased dispersal for the Port Stephens and Jervis Bay populations. These results are similar to those reported in several other embayment populations, which indicate that dispersal of females occurs less frequently than for males (Krützen et al. 2004; Sellas et al. 2005; Bilgmann et al. 2007a). By remaining in their natal habitats, females can potentially increase their reproductive fitness through greater familiarity with local prey resources and opportunities to associate with familiar females as a collective defence mechanism against predators and coercing males (Connor et al. 2000; Möller and Beheregaray 2004). Conversely, there was no evidence for male-biased dispersal for communities along the continuous coastal habitat (Eden, Hunter coast and Ballina). These results are also similar to other studies in which bottlenose dolphin populations sampled in homogenous environments are connected through both male and female dispersal (Natoli et al. 2005, 2008; Parsons et al. 2006; Bilgmann et al. 2007a; Quérouil et al. 2007). The consistency of sex-specific dispersal patterns across geographically distant populations demonstrates the significant environmental influences on a highly mobile, generalist predator.

Implications for conservation management: the problem of losing socially structured groups

Identifying patterns of population structure with various analytical approaches has provided further insights into factors influencing genetic structuring of bottlenose dolphin populations along the coast and, in turn, supplied valuable information for the management of local communities. *T. aduncus* are rarely sighted further than a few kilometres offshore from the NSW coast. Their dependence to shallow, coastal habitats invariably increases their susceptibility to anthropogenic disturbances, such as inshore development, aquaculture and tourism activities. The Port Stephens and Yamba embayment populations are particularly vulnerable given their relatively small population sizes (Möller et al. 2002; Fury and Harrison 2008), lower levels of genetic diversity and limited dispersal (this study). In particular, the eastern Port Stephens community is subject to intensive levels of dolphin watching activity (Allen et al. 2007). Changes to dolphin behaviour and group stability (rates of fission–fusion) as a result of these repeated disturbances may have negative long-term consequences for the population (Allen 2005). Indeed, short-term impacts as a result of tourism activity have been linked to long-term changes in relative abundance of dolphins in Shark Bay, Western Australia (Bejder et al. 2006). Isolated bottlenose dolphin populations exposed to relatively low levels of commercial dolphin watching tourism in Fiordland, New Zealand, have also experienced recent population declines pointing toward unsustainable levels of anthropogenic disturbance (Lusseau et al. 2006; Currey et al. 2008). This is particularly concerning for the Port Stephens dolphin population where the level of dolphin-watching tourism is substantially higher than in both Shark Bay and Fiordland. These findings are exacerbated by the results of asymmetrical migration patterns between Port Stephens and Hunter coast communities as a consequence of either social barriers created by resident dolphins in the port or, potentially, the increased tolerance of some resident dolphins to anthropogenic activities within Port Stephens. Largely unregulated commercial dolphin watching tours, recreational and commercial fishing activities combined with pollution through escalating use of inshore waterways are also occurring at several other NSW localities. Identifying and reducing potential threats should be prioritised given the genetic distinctiveness of most dolphin communities and the significant impacts these threats have had on other cetacean populations (Stone and Yoshinaga 2000; Shaughnessy et al. 2003; Kemper et al. 2005).

Finally, preserving levels of genetic diversity through the protection of local communities will help support genetic connectivity among NSW dolphin populations. The distribution of discrete communities along the NSW coast,

each governed internally by their own dynamics, yet influenced by the demographic processes of nearby populations through the dispersal of individuals, may indicate a metapopulation model of structure (as defined by Kritzer and Sale 2004). As observed in this study, the rate and spatial scale of dispersal events in a metapopulation varies significantly and should be taken into consideration during the design of Marine Protected Areas in NSW. The disappearance of local, yet interconnected, communities may further the divergence of small embayment communities, leading to a possible reduction in genetic variation through inbreeding. Although our estimates of recent migration rates suggest that re-colonisation of available habitats is likely to occur, a community's culture—containing individual knowledge of the environment and vertically transmitted specialisations that enable individuals to forage effectively in their habitat—may be lost with the disappearance of socially structured groups (Whitehead et al. 2004). In Port Stephens for instance, distinct patterns of social behaviour have been observed between 'eastern' dolphins, which live in a typically marine environment, and 'western' dolphins that inhabit areas dominated by estuarine processes (Wiszniewski et al. 2009). Nichols et al. (2007) also demonstrated that re-colonisation of suitable habitat by bottlenose dolphins may not always occur if the metapopulation as a whole is in decline. Taken together, the recovery of dolphin communities to original numbers or the re-colonisation of available habitats is likely to be severely hampered if the cause of the population decline is not firstly identified and managed accordingly (Irwin and Würsig 2004; Nichols et al. 2007). In light of these considerations, the small Port Stephens and Yamba dolphin communities should be considered as discrete management units and protected by minimising potential threats using a precautionary management approach. Furthermore, long-term population monitoring with additional research concentrated on obtaining baseline population data and quantifying specific impacts of human activities in different areas is warranted to help maintain population numbers and connectivity.

Appendix

See Table 6.

Table 6 Levels of genetic variability in NSW bottlenose dolphins by locus and population

Locus		ED ($N = 22$)	JB ($N = 22$)	West PS ($N = 17$)	East PS ($N = 42$)	NC ($N = 19$)	BI ($N = 20$)	FOR ($N = 9$)	YAM ($N = 20$)	BB ($N = 14$)
EV1	AR	4.939	4.22	3.905	3.462	3.359	4.573	3	6.041	4.514
	H_O	0.762	0.591	0.667	0.659	0.444	1.000	0.667	0.875	0.714
	H_E	0.732	0.710	0.637	0.590	0.587	0.764	0.712	0.839	0.765
EV37	AR	3.258	4.415	3.241	3.761	4.512	4.121	3.799	6.892	4.436
	H_O	0.364	0.727	0.647	0.512	0.632	0.650	0.556	0.947	0.769
	H_E	0.428	0.774	0.627	0.517	0.592	0.704	0.673	0.851	0.646
KW2	AR	4.775	4.501	5.351	6.194	6.041	5.946	4.843	5.229	5.32
	H_O	0.591	0.700	0.765	0.833	0.722	0.800	0.625	0.850	0.786
	H_E	0.735	0.746	0.822	0.855	0.863	0.853	0.775	0.826	0.828
KW12	AR	4.235	4.722	2.54	3.566	4.452	3.499	2.971	2.552	2.951
	H_O	0.818	0.818	0.375	0.690	0.778	0.474	0.667	0.300	0.500
	H_E	0.732	0.734	0.383	0.691	0.770	0.697	0.582	0.392	0.669
MK5	AR	2.866	2.832	2.288	2.045	2.751	3.493	2.877	2.958	3.109
	H_O	0.524	0.500	0.333	0.195	0.500	0.526	0.444	0.600	0.857
	H_E	0.582	0.547	0.352	0.226	0.490	0.572	0.464	0.673	0.585
MK6	AR	3.198	3.481	3.241	3.38	3.93	3.496	3.569	3.357	4.244
	H_O	0.545	0.500	0.588	0.537	0.526	0.632	0.889	0.389	0.846
	H_E	0.560	0.660	0.627	0.555	0.580	0.589	0.660	0.611	0.714
MK8	AR	4.382	3.966	2.617	2.266	3.871	3.994	3.873	3.654	5.507
	H_O	0.773*	0.682	0.750	0.643	0.556	0.800	0.556	0.600	0.714
	H_E	0.763	0.647	0.603	0.527	0.587	0.690	0.752	0.628	0.836

N number sampled, *AR* allelic richness, H_O observed heterozygosity, H_E expected heterozygosity

Asterisks denote loci with significant deviation from Hardy Weinberg equilibrium

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