Local population structure and abundance of Hector's dolphins off Kaikoura – 2014 and 2015

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Summary

Hector's dolphins (*Cephalorhynchus hectori hectori*) are distributed discontinuously around the South Island of New Zealand, with genetically differentiated regional populations along the east, west and south coasts. Fine-scale assessments of local population structure are needed to better understand the role of corridors and local dispersal on the maintenance or loss of connectivity in Hector's dolphins. Here we report on a two-year project to better characterise the identity, population structure and abundance of Hector's dolphins near Kaikoura using biopsy samples for DNA profiling, including microsatellite genotyping at up to 18 loci, sequencing of mtDNA control region haplotypes and sex identification.

A total of 15 dedicated small-vessel surveys were conducted in 2014 and 2015 to collect biopsy samples from Hector's dolphins in two local populations, north (Kaikoura-North) and south (Kaikoura-South) of the Kaikoura Canyon. In 2014, nine surveys were conducted on eight days between 22 April and 2 May, during which 30 groups of Hector's dolphins were encountered (average group size = 4.2) and 86 biopsy samples were collected (n = 49 from Kaikoura-North and n = 37 from Kaikoura-South). In 2015, six surveys were conducted between 21 April and 3 May, during which 31 groups of Hector's dolphins were encountered (average group size = 3.6) and 71 biopsy samples were collected (n = 59 from Kaikoura-North and n = 12 from Kaikoura-South).

DNA profiles were obtained from all but one of the biopsy samples, and used to identify 117 individuals (80 from Kaikoura-North and 37 from Kaikoura-South). A slight, but non-significant female bias was found in both populations and years. Fourteen mtDNA haplotypes were identified, four of which were newly described for the species (Cc and AC, AD and AE). Significant genetic differentiation was found between each pairwise comparison of Kaikoura-North, Kaikoura-South, and nearby Cloudy Bay for both mtDNA and microsatellites. Interestingly, Kaikoura-South showed a similar level of genetic differentiation from both the adjacent Kaikoura-North population and the geographically more distant Cloudy Bay, while Kaikoura-North showed very low genetic differentiation from Cloudy Bay. No genotype matches were identified between the three populations, however, one individual from Kaikoura-North (Che14KK61) was previously sampled there as CheKK0308 in April 2003. One individual (Che14KK80) sampled in Kaikoura-North showed genetic evidence of having paternal immigrant ancestry from Kaikoura-South.

Using a two-sample capture-recapture model, the abundance of Hector's dolphins age 1^+ in Kaikoura-North was estimated to be N_{1+} = 314 (95% CL: 216-483; CV = 0.32), based on five annual genotype recaptures between the 43 individuals sampled in 2014 and 42 individuals sampled in 2015. The abundance of Hector's dolphins age 1^+ in Kaikoura-South was estimated to be N_{1+} = 102 (95% CL: 68-175; CV = 0.4), based on two annual genotype recaptures between the 30 individuals sampled in 2014 and nine individuals sampled in 2015. The abundance of Hector's dolphins age 1^+ in the combined study areas encompassing both the northern and southern distributions was estimated to be N_{1+} = 480 (95% CL: 342-703; CV = 0.29), based on the total of seven annual genotype recaptures between the 73 individuals sampled in 2014 and 51 individuals sampled in 2015.

Our work confirms the hypothesis, based on previous photo-identification observations that Hector's dolphins north and south of the Kaikoura Canyon belong to demographically and genetically differentiated populations. This represents the first documentation of significant genetic differentiation between directly adjacent populations of Hector's dolphins, and supports the assumption that local oceanographic features can represent semi-permeable barriers to dispersal and gene flow. Although Kaikoura-North is geographically adjacent to Kaikoura-South, it showed a closer genetic relationship to the more distant Cloudy Bay. Therefore, the 'barrier' presented by the Kaikoura Canyon, appears strong enough to create a disjunction in the overall isolation by distance pattern observed in the regional populations. Our work demonstrates the value of fine-scale genetic sampling for identifying population boundaries of Hector's dolphins and for characterising habitat necessary to maintain connectivity between local populations. While our results suggest that very few Hector's dolphins disperse between Cloudy Bay, Kaikoura-North and Kaikoura-South on a per-generational time-scale, this low level of connectivity is likely to be important for maintaining the genetic diversity and evolutionary potential of these relatively small local populations.

Introduction

Hector's dolphins (*Cephalorhynchus hectori hectori*) are distributed discontinuously around the South Island of New Zealand, with regional populations along the east, west and south coasts. Previous descriptions of mitochondrial (mt) DNA diversity provided evidence of strong genetic differentiation between these three regional populations (Pichler *et al.* 1998, Pichler and Baker 2000, Hamner *et al.* 2012). A meta-analysis of mtDNA and microsatellite loci confirmed the isolation of these regional populations and suggested further subdivision between local populations within regions (Hamner *et al.* 2012). Of particular importance for management was the conclusion that the protection of corridors, as well as local populations, is required to maintain the 'stepping stone' pattern of dispersal and gene flow linking adjacent local populations and the very rare migration events linking regional populations (Hamner *et al.* 2012). Fine-scale assessments of local population structure are needed to better understand the role of corridors and local dispersal on the maintenance or loss of connectivity in Hector's dolphins.

Photo-identification observations of Hector's dolphins off Kaikoura suggested that individuals north and south of the Kaikoura Canyon belong to demographically independent populations (Weir and Sagnol 2015). Here we report on the results of a two-year project to better characterise the genetic identity, population structure and abundance of Hector's dolphins near Kaikoura using biopsy samples for DNA profiling. The objectives were:

- To individually identify Hector's dolphins by DNA profiling, including microsatellite genotyping at up to 18 loci, sequencing of mtDNA control region haplotypes and sex identification
- To search for replicate samples (genotype recaptures) from north and south of the Kaikoura Canyon
- To identify long-term residents from genotype matches between Hector's dolphins sampled in Kaikoura in 2014-2015 and individuals (n = 7) sampled there in 2003
- To identify potential long-range dispersal by matching genotypes of Hector's dolphins sampled in Kaikoura in 2014 and 2015 to a large collection of genotypes (n = 147 individuals) from Cloudy Bay, approximately 120 km away
- To identify genotype recaptures between 2014 and 2015, and use these to estimate the abundance of the populations north and south of the Kaikoura Canyon
- To test for genetic differentiation between dolphins sampled north and south of the Kaikoura Canyon and in Cloudy Bay using both mtDNA and microsatellite loci
- To identify potential migrants across the Kaikoura Canyon or to/from Cloudy Bay using genotype assignment

Methods

Small-vessel surveys, photo-identification and biopsy sampling

Surveys of Hector's dolphins were conducted in the Kaikoura area aboard the M/V *Titi*, a 6 m, aluminium monohull (Stabicraft 2050 Supercab), powered by a 200 hp, four-stroke outboard engine (Yamaha). A high-speed digital SLR camera (Nikon D90 with 70-300 mm lens) was used to photograph the dorsal fins of all Hector's dolphins in proximity to the research vessel during each encounter. Skin biopsy samples were collected with a Paxarms modified veterinary capture rifle (Krützen *et al.* 2002) and 'dolphin' biopsy tips (approximately 6 mm in length and 4 mm in diameter). Calves, approximately one-half or less the size of an adult (assumed to be <1 year old; Webster et al. 2010), were excluded from

biopsy sampling. Behavioural reactions to biopsy darting were judged according to the classification system presented by Tezanos-Pinto & Baker (2012). The biopsy samples were stored in 70% ethanol and transferred to the University of Auckland for sub-sampling and archiving in the New Zealand Cetacean Tissue Archive. Sub-samples were transferred to the Cetacean Conservation and Genomics Laboratory at Oregon State University for DNA extraction and genotyping.

DNA profiling, individual identification, and sex ratio

Total cellular DNA was extracted using a standard phenol/chlorofom/isoamyl (PCI) protocol (Sambrook *et al.* 1989), as modified for small samples by Baker *et al.* (1994). DNA profiling included a standard set of genetic markers used previously for research on Hector's and Māui dolphins: sex, mitochondrial (mt) DNA control region haplotype and microsatellite genotype (*e.g.*, Pichler 2002, Hamner *et al.* 2012, Oremus *et al.* 2012, Baker *et al.* 2013, Hamner *et al.* 2014a, Hamner *et al.* 2014b). Genetic sex identification and mtDNA control region sequencing were carried out according to Hamner *et al.* (2012). The sex ratio for the individuals sampled was compared to an expected 1:1 ratio using a two-tailed exact binomial test. Geneious Pro 6.1.8 (Biomatters Ltd.) was used to assign mtDNA haplotypes based on alignment with 576 bp reference sequences for the 34 described Hector's dolphin haplotypes and single Māui dolphin haplotype (Pichler *et al.* 1998, Pichler and Baker 2000, Pichler 2002, Hamner 2008, Hamner *et al.* 2013, Hamner *et al.* 2014a, unpublished data).

Eighteen microsatellite loci were amplified individually in 10 μL PCR reactions. For loci with M13-tagged labels (see Table 1), each reaction contained 1x PCR II buffer, 2.5 mM $MgCl_2$, 0.04 μM forward primer with M13 tag, 0.4 μM reverse primer, 0.4 μM M13-tagged fluorescent label, 0.2 mM dNTP, 20 mg/mL bovine serum albumin (BSA), 0.25 units Platinum *Taq* (Invitrogen) and 10–20 ng/μL DNA template. These were amplified using the thermocycling profile of Cunha and Watts (2007) with modifications to the annealing temperature as specified in Table 1. For all other loci, each 10 μL PCR reaction contained 1x PCR II buffer, 2.5 mM $MgCl_2$, 0.4 μM each primer, 0.2 mM dNTP, 0.125 units Platinum *Taq* (Invitrogen) and 10–20 ng/μL DNA template. These were amplified using the following thermocycling profile: 93°C for 2 min; (92°C for 30 s, T_A for 45 s, 72°C for 50 s) x 15; (89°C for 30 s, T_A for 45 s, 72°C for 50 s) x 20; 72°C for 3 min, with the annealing temperatures (T_A) stated in Table 1. Up to six loci of different size and/or label that were amplified from the same individual were co-loaded for sizing by an ABI 3730XL Genetic Analyzer (Applied Biosystems). Geneious Pro 6.1.8 (Biomatters Ltd.) was used to bin and visually verify the resulting size peaks. Each amplification and sizing run included a negative control to check for contamination and four to eight internal control samples to standardize allele binning with previous genotyping runs. Approximately 10% of the 157 samples (n = 16) were randomly selected for replicate genotyping to estimate genotyping error by dividing the number of incongruent allele calls by the total number of alleles repeated, as recommended by Bonin *et al*. (2004). GenAlEx 6.5 (Peakall and Smouse 2006) was used to calculate the probability of identity (P_{ID}) and probability of identity for siblings (P_{IDsib}) for each locus, as well as for all loci combined.

Microsatellite genotypes were compared using CERVUS 3.0.3 (Kalinowski *et al.* 2007) to identify individuals and re-samples of the same individual. This comparison also included genotypes for seven individuals sampled alive off Kaikoura in 2003 (11 loci; Hamner *et al.* 2012) and 147 individuals sampled in Cloudy Bay in 2011-12 (Hamner *et al.* 2013). Initial comparisons allowed for mismatching of up to five loci ('relaxed matching') to prevent false exclusion due to genotyping error. Relaxed matches were visually examined for potential

allelic dropout, as well as matching sex and mtDNA haplotype. After review, and correction if necessary, samples with identical genotypes or apparent allelic dropout at one locus were accepted as resamples of the same individual, based on a low probability of identity (P_{ID}) and probability of identity for siblings (P_{IDsib}). Observed and expected heterozygosity, F_{IS}, and a test for deviations from Hardy-Weinberg equilibrium were calculated in GenAlEx v6.5 (Peakall and Smouse 2006). Micro-Checker v.2.2.3 (Van Oosterhout *et al.* 2004) was used to assess the presence of null alleles.

Genotype capture-recapture abundance

Capture histories for individuals identified by the DNA profiles were assembled based on genotype captures in the two occasions, 2014 and 2015. Abundance and the coefficient of variation (CV, a measure of precision) were calculated using the Lincoln-Petersen estimator with Chapman correction (Chapman 1951) and 95% confidence limits (CL) were calculated according to Chao's (1989) method.

Genetic differentiation and population assignment

To discount any significant change in the population of dolphins using the study areas between our two sampling occasions, we used the program Arlequin v3.5.1.2 (Excoffier and Lischer 2010) to assess the genetic differentiation (F_{ST}) between the samples collected in the two occasions based on both mtDNA and microsatellite data. Although the highly diverse microsatellite locus PPHO104 is useful for individual identification, it appears to be influenced by selection (Hamner 2014) and was therefore excluded from this and the following analyses, which assume neutral evolution. Pairwise F_{ST} values calculated from mtDNA and 17 microsatellites in Arlequin v3.5.1.2 (Excoffier and Lischer 2010) were used to assess the genetic differentiation among the two hypothesized populations north and south of the Kaikoura Canyon (*i.e.*, Kaikoura-North and Kaikoura-South) and the nearby population in Cloudy Bay. Higher F_{ST} values indicate greater genetic differentiation between populations, and $p < 0.001$* indicates a probability of less than 0.1% that the observed F_{ST} would be produced by chance.

To identify any potential migrants between Kaikoura-North, Kaikoura-South and Cloudy Bay, the Bayesian assignment method of *Structure* (Pritchard *et al.* 2000) was used to assess the likely population of origin for each individual based on how well its genotype fits with the others sampled in that area. Following (Hamner *et al.* 2012), the ''Use PopInfo'' option (G = 0) was applied to run 10^6 Markov chain Monte Carlo (MCMC) replicates following a burn-in of 10^5 for K = 3 populations (*i.e.*, Kaikoura-North, Kaikoura-South, and Cloudy Bay).

The program *Structure* was also run without *a priori* population information to investigate the potential for any cryptic population structure. Using the no admixture and correlated allele frequency models, without the "Use PopInfo" option, six iterations of 10^6 Markov chain Monte Carlo replicates following a burn-in of 10^5 were run for K = 1 - 5 inferred populations. The most likely number of populations (K) was determined by examining the log likelihood values, LnP(K), in addition to ΔK (Evanno *et al.* 2005) as calculated by Structure Harvester v0.6.94 (Earl and vonHoldt 2011).

Results

Surveys, sample collection, and other cetacean sightings

A total of 15 surveys were conducted, with nine between 22 April and 2 May 2014 and six between 21 April and 3 May 2015 (Table 2). An additional survey was terminated because of

engine problems (27 April 2014). The vessel was launched from and returned to South Bay Marina each day, with surveys covering the waters north and south of Kaikoura Peninsula (Table 2). In 2014, 30 groups of Hector's dolphins were encountered, with an average group size of 4.2 (range: 1 – 10; Figure 1; Table 2). Similarly in 2015, 31 groups were encountered, but with an average group size of 3.6 (range: 1-12; Figure 1; Table 2). Due to weather conditions and sea state, less time was spent on the water in 2015 and fewer dolphins were encountered. This resulted in fewer biopsy samples and photographs in this year, particularly in the area south of the Kaikoura Canyon.

A total of 157 biopsy samples were collected from Hector's dolphins during the surveys, of which 108 were north of the Kaikoura Canyon (Kaikoura-North) and 49 were south of the Kaikoura Canyon (Kaikoura-South). Behavioural reactions to biopsy darting ranged from 'no visible reaction' (Level 0) to 'flinch/splash/short burst reaction' (Level 2), according to the classification system presented by Tezanos-Pinto & Baker (2012). There was one case in each year where one dart did not dislodge from a dolphin and was not recovered. There were no unusual behavioural responses in these cases.

A total of 492 photographs (2014 n = 402; 2015 n = 90) were taken during encounters with Hector's dolphins. These were transferred to the Kaikoura Ocean Research Institute's photo-ID collection for sorting, cataloguing, and matching.

In addition to Hector's dolphins, a pod of four killer whales was sighted and photographed on 23 April 2014 and three blue whales were sighted and photographed on 24 April 2014 (Olson *et al.* 2015). No biopsy samples were collected from these notable non-target species.

DNA profiling, individual identification and sex ratio

DNA profiles were obtained from all but one of the 157 biopsy samples collected. The exception was Che14KK26, an atypically small biopsy sample that yielded DNA of very low quantity and quality, and failed to amplify for all but the mitochondrial locus. Therefore, 156 samples (Kaikoura-North n = 108, Kaikoura-South n = 48) were included in the following analyses. Based on 576 bp of the mtDNA control region, 14 haplotypes were identified (Table 3 and Figure 2), of which ten were previously described in Hector's dolphins (Pichler *et al.* 1998, Pichler and Baker 2000, Pichler 2002, Hamner 2008, Hamner *et al.* 2012, Hamner 2014, Hamner *et al.* 2014a, unpublished data) and four were newly described (Cc, AC, AD, and AE). These new haplotypes each differed from one to three known haplotypes by a single base pair.

Each sample was genotyped for up to 18 microsatellite loci, with an average of 17.8 loci per sample (Table 4). The repeated genotyping of 16 samples for 15 loci (480 alleles) resulted in identical genotypes for all but one sample, which was determined to be a processing error (*i.e.*, Che14KK22 substituted for Che14KK21). No evidence of null alleles was found. Considering all loci, the probability of identity (P_{ID}) was 1.1×10^{-13} and probability of identity for siblings (P_{IDsib}) was 9.5×10^{-6} (Table 4). Given this low probability of a match by chance, we assumed that unique genotypes represent individual dolphins and that samples with matching genotypes, allowing for one apparent allelic dropout, were in fact replicate samples of the same individual. Sex and mtDNA haplotype were subsequently compared and agreed for all of the genotype matches, with one exception where sex was uncertain. Given the low probability of two individuals having matching genotypes at the 14 loci for which both had data ($P_{ID} = 8.37 \times 10^{-12}$; $P_{IDsib} = 1.39 \times 10^{-4}$), the genotype was only represented once in analyses.

From the 156 samples with microsatellite genotypes, a total of 117 individuals were identified (Kaikoura-North $n = 80$, Table 5; Kaikoura-South $n = 37$; Table 6). Most individuals ($n = 85$) were sampled once during the two field seasons, with $n = 26$ sampled twice, $n = 5$ sampled three times and $n = 1$ sampled four times. Comparison of genotypes with the seven individuals sampled in April 2003 identified one genotype recapture: sample Che14KK61 was a match to CheKK0308 (Hamner *et al.* 2012). The overall sex ratio of individuals reflected a slight, but non-significant female bias ($p = 0.163$; Table 7).

Abundance north and south of the Kaikoura Canyon

Based on the genotype recapture histories, the abundance of Hector's dolphins age 1^+ in the Kaikoura-North study area was $N_{1+} = 314$ (95% CL: 216 – 483) and in the Kaikoura-South study area it was $N_{1+} = 102$ (95% CL: 68 – 175; Table 8, Sup. Mat. 1). The abundance of Hector's dolphins age 1^+ in the total study area encompassing both the northern and southern populations was calculated to be $N_{1+} = 480$ (95% CL: 342-703; Table 8 Sup. Mat. 1).

Temporal genetic differentiation

No genetic differentiation was found between the individuals sampled in Kaikoura-North in 2014 and those sampled in 2015 (mtDNA $F_{ST} = 0.01$, $p = 0.261$; microsatellite $F_{ST} = 0$, $p = 0.919$). This is consistent with the assumption that the same genetic population was present in the area during both sampling occasions. A similar result was found for Kaikoura-South based on microsatellites ($F_{ST} = 0$; $p = 0.649$), but the mtDNA results suggested some differences between the annual samples ($F_{ST} = 0.11$, $p = 0.063$). This is likely an artifact resulting from the low sample size of n = 9 individuals representing Kaikoura-South in 2015.

Spatial genetic differentiation and population assignment

Significant genetic differentiation was found between each pairwise comparison of Kaikoura-North, Kaikoura-South, and Cloudy Bay for both mtDNA and microsatellites (Table 9). Interestingly, Kaikoura-South showed a similar level of genetic differentiation from both the adjacent Kaikoura-North population and the geographically more distant Cloudy Bay, while Kaikoura-North showed very low genetic differentiation from Cloudy Bay (Table 9).

The *Structure* assignment analysis (*i.e.*, 'Use PopInfo' option) showed that most individuals were assigned to their sampling location with high membership coefficients (Figure 3). However, Che14KK80, a female sampled in Kaikoura-North, was cross-assigned to Kaikoura-South with a membership coefficient of $q = 0.7$ (Figure 3). Che14KK80's maternally inherited mtDNA haplotype of Ia has not been detected in Kaikoura-South, suggesting that this individual is likely to be the offspring of a Kaikoura-North mother and an immigrant father from Kaikoura-South.

When *Structure* was used to identify the number of populations without *a priori* information (*i.e.*, without the "Use PopInfo" option), the highest probability was for K = 1 population. This is consistent with the limitations of the method implemented by *Structure* when differentiation between populations is weak; *e.g.*, *Structure* is unlikely to detect populations that differ with $F_{ST} < 0.02$ (Hubisz *et al.* 2009).

Discussion

Our work confirms the hypothesis, based on previous photo-identification observations (Weir and Sagnol 2015), that the Hector's dolphins north and south of the Kaikoura Canyon belong

to demographically and genetically differentiated populations. This represents the first documentation of significant genetic differentiation between directly adjacent populations of Hector's dolphins and supports the assumption that local oceanographic features can represent semi-permeable barriers to dispersal and gene flow.

Semi-permeable barriers to dispersal and gene flow

Water depth, particularly in excess of 100 m, has been suggested as a factor limiting the distribution of Hector's dolphins and their movements across areas like Fiordland and Cook Strait (Bräger *et al.* 2003, Slooten *et al.* 2006). Not far south of Kaikoura Peninsula, the head of the Kaikoura Canyon is located 500 m from the shore (Lewis and Barnes 1999). The canyon's depth increases sharply, exceeding 100 m within as little as 1 km of shore, and reaching depths of 1200 m at points along its 60 km length (Lewis *et al.* 1998, Lewis and Barnes 1999). The configuration of the Kaikoura Canyon provides only a narrow inshore corridor of shallow water to facilitate north/south movements by Hector's dolphins if they are to avoid crossing depths greater than 100 m.

Hector's dolphins exhibit an overall genetic pattern of isolation by distance, whereby step-wise gene flow links adjacent local populations within larger regions, and greater geographic distance results in greater genetic differentiation (Pichler 2002, Hamner *et al.* 2012). Previous studies did not detect significant differentiation between directly adjacent local populations, including Cloudy Bay and Kaikoura (Pichler 2002, Hamner *et al.* 2012). While these previous studies provided critical information about the larger-scale population structure of the species, they did not have the sample sizes necessary to detect fine-scale population structure or capture all of the genetic diversity present in all areas. For example, on the east coast of the South Island, samples were pooled over 20 years to achieve low, but useful, sample sizes (n = 13 – 34) for local populations that were defined by sampling locations spanning approximately 75 – 175 km of coastline. In contrast, the larger number of individuals sampled over a short period of time and across a small geographic area facilitated our current detection of differentiation among Cloudy Bay and each of the two Kaikoura populations. Interestingly, the 'barrier' presented by the Kaikoura Canyon, appears strong enough to create a disjunction in the overall isolation by distance pattern. Although Kaikoura-North is geographically adjacent to Kaikoura-South, it showed a closer genetic relationship to the more distant Cloudy Bay.

The patterns of genetic diversity and population connectivity in Hector's dolphins are likely caused by a combination of oceanographic and distance-related factors. For comparison, the two South Coast populations of Te Waewae and Toetoe Bays are separated from each other by approximately 100 km and show a greater degree of differentiation (mtDNA F_{ST} = 0.136, microsatellite F_{ST} = 0.043, $p < 0.05$; Hamner *et al.* 2012) than the two populations on either side of the Kaikoura Canyon (nearshore depth >100m for ~12 km). This suggests that a more isolating restriction in gene flow will result from a large distance with no 'stepping-stones' than from a very narrow corridor of shallow water. On the other hand, a more continuous distribution of dolphins without depth-related barriers, such as the 55 km between the proximate boundaries of Cloudy Bay and Kaikoura-North (MacKenzie and Clement 2014), appears to facilitate a higher, albeit still low, level of gene flow.

More complete description of genetic diversity

The increased sampling of Hector's dolphins off Kaikoura by the current project also allowed us to capture a more complete picture of their genetic diversity. This included the identification of four mtDNA haplotypes that had not been previously described in this or

other areas. Two of these haplotypes were detected only in Kaikoura-North, while the other two were detected only in Kaikoura-South. Although these haplotypes were not detected by the recent work in Cloudy Bay to the north (Hamner *et al*. 2013), additional intensive sampling would be required to investigate their presence in other Hector's dolphin populations. All four novel haplotypes were detected at very low frequencies, comprising 2-5% of the individuals sampled in their respective populations (*i.e.,* one to four individuals). The pattern whereby populations share several common mtDNA haplotypes, while each retains a few unique haplotypes that occur at low frequencies, is typical of populations that are experiencing low levels of female gene flow per generation.

Low local abundance and conservation implications

Step-wise genetic connectivity is likely playing an important role in maintaining genetic diversity and evolutionary potential in the subspecies, given the relatively low abundances that characterise the local populations along continuous distributions. Genotype recapture analysis has now been used to estimate the abundance of local populations at three points along a continuous distribution of Hector's dolphins: Cloudy Bay (Hamner *et al.* 2013), Kaikoura-North and Kaikoura-South. Although the lower sample sizes resulted in a less precise estimate of abundance for Hector's dolphins off Kaikoura compared to Cloudy Bay, the results are sufficient to conclude that each population numbers only a few hundred individuals. Our abundance estimates for Hector's dolphins off Kaikoura are also consistent with those obtained using alternative methods that focused on slightly different study areas. Photo-identification recapture resulted in an abundance estimate of 304 (95% CL = 211-524) for the coastal area between the Hapuku River and Haumuri Bluffs (Weir and Sagnol 2015), which centers on the study area of our current work and includes part of both populations we identified. Aerial line-transect surveys were used to estimate a summer abundance of 358 (95% CL = 129-999) for an area also bordered by the Hapuku River, but extending further south than our Kaikoura-South study area to the northern edge of Pegasus Bay near Motunau (MacKenzie and Clement 2014).

In isolation, such small populations would be at risk of experiencing negative effects associated with the loss of genetic diversity, increase in inbreeding due to non-random mating, and less efficient operation of natural selection. However, as part of a larger meta-population, low levels of gene flow between adjacent populations can maintain genetic diversity, while facilitating beneficial local adaptations (*e.g*., Allendorf *et al.* 2013). The maintenance of a corridor for gene flow between Hector's dolphins north and south of the Kaikoura Canyon is likely to be aided by the recently established Hikurangi Marine Reserve and Kaikoura Whale Sanctuary. The Hikurangi Marine Reserve protects approximately 2 km along the shore north of Goose Bay, and extends out to 23.4 km, encompassing an area of 10,416 hectares around the deep waters of the Kaikoura Canyon. It does not allow mining, fishing or harvesting of any kind. Furthermore, the Kaikoura Whale Sanctuary, which covers 45 km to the north and south of the Kaikoura Peninsula and extends 56 km out to sea, prohibits high-level seismic surveys.

Additional work for Kaikoura-South

Additional work is needed to better characterise the Kaikoura-South population. The low sample size obtained for this population, particularly in 2015, resulted from the challenges of sighting dolphins among the large swells that prevailed during the field season and the constraints of safely approaching dolphins sighted in the surf break. Interestingly, Haumuri Bluffs was a hotspot for sightings in 2014, but only a small number of dolphins were encountered there in 2015. Although the mtDNA data appeared to suggest that the Hector's

dolphins sampled in Kaikoura-South in 2014 showed genetic differentiation from the ones sampled there in 2015, this is likely an artifact due to the very low sample size of nine individuals in 2015. This conclusion is consistent with the lack of temporal differentiation indicated by the microsatellite analysis. Given the relatively low abundance estimate for Hector's dolphins in the Kaikoura-South study area (*N*=102, 95% CL 68-175), this area might require increased effort to find dolphins if they are present at low density across the area. Additional surveys and biopsy samples from Kaikoura-South would allow a more robust abundance estimate, as well as a better understanding of the spatial use patterns by the local Hector's dolphins. Furthermore, conducting similar studies of the local populations to the south of this area would provide information on the degree of connectivity, or isolation, of the small Kaikoura-South population with its southern neighbor, as well as improving our understanding of connectivity among Hector's dolphins along the entire east coast, South Island region.

Conclusion

Our study demonstrates the value of fine-scale genetic sampling for identifying population boundaries of Hector's dolphins, improving descriptions of genetic diversity, and characterising habitat necessary to maintain connectivity between local populations. While our results suggest that very few Hector's dolphins disperse between Cloudy Bay, Kaikoura-North and Kaikoura-South, on a per-generational time-scale, this low level of connectivity is important for maintaining the genetic diversity and evolutionary potential of these populations, and ultimately the subspecies.

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Table 1. Amplification conditions and primer sources for microsatellite loci genotyped for Hector's dolphins off Kaikoura (T_A = annealing temperature).

Locus	Primer Label	T_A (ºC)	Primer Source
GT211	6-FAM	50	Bérubé *et al.* 2000
GT575	6-FAM	50	Bérubé *et al.* 2000
KWM9b	6-FAM	50	Hoelzel *et al.* 2002
PPHO142	VIC	55	Rosel *et al.* 1999
KWM12a	VIC	55	Hoelzel *et al.* 1998
SGUI03	NED-M13	57	Cunha and Watts 2007
EV104	6-FAM	45	Valsecchi and Amos 1996
EV94	6-FAM	55	Valsecchi and Amos 1996
SGUI16	6-FAM-M13	57	Cunha and Watts 2007
MK5	VIC-M13	55	Krützen *et al.* 2001
EV1	NED	45	Valsecchi and Amos 1996
415/416	NED	45	Schlotterer *et al.* 1991
PPHO110	6-FAM	50	Rosel *et al.* 1999
PPHO104	6-FAM	50	Rosel *et al.* 1999
GT23	VIC	55	Bérubé *et al.* 2000
EV14	VIC	60	Valsecchi and Amos 1996
SGUI06	VIC-M13	57	Cunha and Watts 2007
SGUI17	NED-M13	60	Cunha and Watts 2007

Table 2. Vessel surveys conducted to collect biopsy samples from Hector's dolphins in the Kaikoura area in 2014 and 2015. *Note: Survey direction is from South Bay Marina on Kaikoura Peninsula, not Kaikoura Canyon, therefore a couple 'South' surveys include several samples from the Kaikoura-North population.

Year	Survey Number	Date	Departure	Return	Time (hours)	Survey Direction*	Groups Encountered	Samples Collected
2014	1	22-Apr-14	9:58	14:20	4.37	North	5	14
	2	23-Apr-14	8:06	11:20	3.23	South	1	16
	3	23-Apr-14	13:16	18:10	4.90	North	3	9
	4	24-Apr-14	9:20	14:54	5.57	South	5	5
	5	25-Apr-14	8:26	10:57	2.52	North	3	0
	6	26-Apr-14	9:46	15:00	5.23	South	4	16
	7	27-Apr-14	8:30	12:45	4.25	North	0	0
	8	1-May-14	9:15	15:20	6.08	North	4	12
	9	2-May-14	8:10	15:20	7.17	North	5	14
	Total				43.32		30	86
2015	1	21-Apr-15	8:30	12:50	4.33	North	6	19
	2	23-Apr-15	7:56	11:15	3.32	North	2	11
	3	24-Apr-15	8:10	13:05	4.92	South	2	4
	4	25-Apr-15	7:30	10:45	3.25	South	1	0
	5	2-May-15	8:00	16:45	8.75	North	11	23
	6	3-May-15	7:55	16:00	8.08	South	9	14
	Total				32.65		31	71
2014-15	15				75.97		61	157

Table 3. Mitochondrial DNA control region (576 bp) haplotype frequencies for Hector's dolphin individuals sampled in Cloudy Bay (2011-12; Hamner *et al.* 2013), and Kaikoura-North and Kaikoura-South in 2014 and 2015. *Four haplotypes were newly identified by the current work.

Haplotype	Cloudy Bay	Kaikoura-North			Kaikoura-South		
	2011-12	2014	2015	2014-15	2014	2015	2014-15
A	12						
Ca	48	22	14	34	10	1	11
Cb1	17	5	7	11	6	5	9
Cb2	7						
Cc*		2	2	4			
D	5		1	1			
E	4				1		1
Ha	1						
Hb	8						
Ia	21	9	12	19			
Ib	3	2	2	4			
Ja	6	1	2	3			
Jb	4						
Jc					3	2	5
P	7						
W	1	1		1	9		9
X	5	1	1	2			
Y	3						
AB	1						
AC*					1		1
AD*			1	1			
AE*						1	1
n Individuals	153	43	42	80	30	9	37
# Haplotypes	17	8	9	10	6	4	7

Table 4. Microsatellite loci genotyped for Hector's dolphins sampled off Kaikoura in 2014 and 2015, including the number of alleles (*k*), probability of identity (P_{ID}), probability of identify for siblings (P_{IDsib}), observed (H_O) and expected (H_E) heterozygosity, a test of deviation from Hardy-Weinberg equilibrium (HWE *P; *p* < 0.05) and inbreeding coefficient (F_{IS}). [a]Loci available for Hector's dolphin samples collected off Kaikoura in 2003.

					Kaikoura-North						Kaikoura-South					
Locus	*n* Samples	*k*	P_{ID}	P_{IDsib}	*n* Indiv.	*k*	H_O	H_E	HWE *p*	F_{IS}	*n* Indiv.	*k*	H_O	H_E	HWE *p*	F_{IS}
GT211	155	5	1.6E-01	4.5E-01	79	5	0.70	0.65	0.490	-0.07	37	4	0.78	0.71	0.328	-0.10
GT575[a]	155	3	3.5E-01	6.0E-01	79	3	0.44	0.46	0.870	0.04	37	3	0.51	0.51	0.306	-0.01
KWM9b[a]	155	7	1.3E-01	4.3E-01	80	6	0.59	0.72	0.000*	0.18	37	7	0.62	0.66	0.634	0.06
PPHO142	156	2	3.8E-01	6.0E-01	80	2	0.46	0.49	0.655	0.05	37	2	0.51	0.50	0.869	-0.03
KWM12a[a]	148	18	6.7E-02	3.7E-01	76	16	0.78	0.81	0.001*	0.05	34	12	0.76	0.78	0.002*	0.02
SGUI03	150	7	1.1E-01	4.0E-01	76	7	0.75	0.75	0.934	0.00	36	6	0.81	0.74	0.537	-0.09
EV104	152	2	5.7E-01	7.6E-01	80	2	0.23	0.20	0.257	-0.13	35	2	0.31	0.37	0.390	0.15
EV94[a]	156	11	8.1E-02	3.8E-01	80	9	0.74	0.78	0.451	0.06	37	9	0.68	0.73	0.998	0.08
SGUI16	156	3	6.1E-01	7.9E-01	80	3	0.21	0.21	0.737	-0.04	37	3	0.27	0.26	0.567	-0.03
MK5[a]	156	3	2.9E-01	5.4E-01	80	3	0.59	0.57	0.817	-0.02	37	3	0.54	0.51	0.802	-0.06
EV1[a]	156	2	4.6E-01	6.8E-01	80	2	0.49	0.43	0.273	-0.12	37	2	0.14	0.17	0.199	0.21
415/416[a]	155	2	6.4E-01	8.0E-01	80	2	0.24	0.21	0.228	-0.14	37	2	0.32	0.27	0.239	-0.19
PPHO110[a]	156	4	1.3E-01	4.2E-01	80	4	0.71	0.74	0.015*	0.04	37	4	0.62	0.68	0.724	0.09
PPHO104[a]	156	60	1.6E-03	2.6E-01	80	55	0.95	0.97	0.078	0.02	37	33	0.92	0.96	0.009*	0.04
GT23[a]	156	4	3.5E-01	6.2E-01	80	4	0.49	0.42	0.596	-0.15	37	4	0.32	0.38	0.893	0.14
EV14[a]	156	4	2.1E-01	4.9E-01	80	3	0.64	0.64	0.623	0.01	37	4	0.65	0.64	0.784	-0.02
SGUI06	156	3	4.1E-01	6.4E-01	80	2	0.40	0.45	0.345	0.11	37	3	0.57	0.46	0.425	-0.24
SGUI17	146	4	3.9E-01	6.4E-01	75	4	0.47	0.40	0.795	-0.16	35	2	0.34	0.41	0.344	0.16
Overall	156	8.0	1.1E-13	9.5E-06	80	7.3	0.55	0.55		-0.02	37	5.8	0.54	0.54		0.01

Table 5. Recapture histories for Hector's dolphins sampled north of the Kaikoura Canyon (*i.e.*, Kaikoura-North). *Che14KK61 was first sampled as CheKK0308 in April 2003.

Individual ID	Sex	mtDNA	22-Apr-14	23-Apr-14	1-May-14	2-May-14	21-Apr-15	23-Apr-15	24-Apr-15	2-May-15	3-May-15
Che14KK01	F	Ca	14KK01; 14KK08				15KK12				
Che14KK02	M	Cb1	14KK02								
Che14KK03	F	Cb1	14KK03								
Che14KK04	F	Ia	14KK04								15KK71
Che14KK05	U	W	14KK05		14KK62						
Che14KK06	M	Ca	14KK06							15KK35	
Che14KK07	F	Ca	14KK07								
Che14KK09	F	Cc	14KK09								
Che14KK10	F	Ia	14KK10								
Che14KK11	F	Ca	14KK11								
Che14KK12	F	Ca	14KK12; 14KK13								
Che14KK14	F	Cb1	14KK14								15KK68;15KK69
Che14KK31	F	Ca		14KK31; 14KK34							
Che14KK32	F	Ia		14KK32							
Che14KK33	F	Ca		14KK33							
Che14KK35	M	Cb1		14KK35							
Che14KK36	F	Cb1		14KK36							
Che14KK37	M	Ca		14KK37							
Che14KK38	F	Ca		14KK38							
Che14KK39	F	Cc		14KK39							
CheKK0308*	F	Ca			14KK61						
Che14KK63	F	Ia			14KK63						
Che14KK64	M	Ia			14KK64						
Che14KK65	F	Ca			14KK65						
Che14KK66	M	Ca			14KK66						
Che14KK67	F	Ia			14KK67		15KK07;15KK09				
Che14KK68	F	Ia			14KK68; 14KK70						
Che14KK69	M	Ca			14KK69						
Che14KK71	F	Ca			14KK71						
Che14KK72	F	Ca			14KK72						
Che14KK73	M	Ca				14KK73					
Che14KK74	M	Ca				14KK74					
Che14KK75	M	Ib				14KK75					
Che14KK76	M	Ca				14KK76					
Che14KK77	M	Ia				14KK77; 14KK78					
Che14KK79	M	Ca				14KK79					
Che14KK80	F	Ia				14KK80					
Che14KK81	F	Ib				14KK81					
Che14KK82	M	X				14KK82					
Che14KK83	F	Ja				14KK83					
Che14KK84	M	Ca				14KK84					
Che14KK85	M	Ca				14KK85					
Che14KK86	M	Ca				14KK86					
Che15KK01	M	Cc					15KK01				
Che15KK02	F	Cb1					15KK02;15KK05				
Che15KK03	M	Cb1					15KK03				
Che15KK04	F	AD					15KK04				
Che15KK06	F	Cb1					15KK06;15KK10				
Che15KK08	F	Ia					15KK08				
Che15KK11	F	D					15KK11;15KK14	15KK21			
Che15KK13	M	Ia					15KK13				
Che15KK15	M	Cb1					15KK15				
Che15KK16	F	Ia					15KK16;15KK17				
Che15KK18	F	Ia					15KK18				
Che15KK19	M	Cc					15KK19				
Che15KK20	F	Ca						15KK20;15KK22			
Che15KK23	F	Ib						15KK23;15KK24			
Che15KK25	F	Ia						15KK25			
Che15KK26	F	Ca						15KK26;15KK28			
Che15KK27	F	Ia						15KK27;15KK30			
Che15KK29	F	Cb1						15KK29			
Che15KK33	F	Cb1							15KK33; 15KK34		
Che15KK36	M	Ia								15KK36;15KK37	
Che15KK38	M	Ia								15KK38	
Che15KK39	M	Ca								15KK39;15KK40	
Che15KK41	M	X								15KK41	
Che15KK42	M	Ib								15KK42	
Che15KK43	F	Ca								15KK43	
Che15KK44	M	Ca								15KK44	
Che15KK45	F	Ia								15KK45;15KK46	
Che15KK47	M	Ca								15KK47	
Che15KK48	M	Ca								15KK48	
Che15KK49	M	Ja								15KK49	
Che15KK50	M	Ja								15KK50;15KK52;15KK53	
Che15KK51	M	Ca								15KK51	
Che15KK54	M	Ca								15KK54	
Che15KK55	F	Ca								15KK55	
Che15KK56	F	Ca								15KK56	
Che15KK57	F	Ca								15KK57	
Che15KK70	F	Ia									15KK70

Table 6. Recapture histories for Hector's dolphins sampled south of the Kaikoura Canyon (*i.e.*, Kaikoura-South).

Individual ID	Sex	mtDNA	22-Apr-14	24-Apr-14	26-Apr-14	24-Apr-15	3-May-15
Che14KK15	F	Cb1	14KK15; 14KK22	14KK42		15KK32	
Che14KK16	M	W	14KK16				
Che14KK17	M	AC	14KK17				
Che14KK18	M	Cb1	14KK18				
Che14KK19	M	W	14KK19				
Che14KK20	M	Ca	14KK20	14KK44			
Che14KK21	M	Ca	14KK21				
Che14KK23	F	Ca	14KK23				
Che14KK24	M	Ca	14KK24				
Che14KK25	M	Ca	14KK25				
Che14KK27	F	W	14KK27	14KK41			
Che14KK28	M	Cb1	14KK28; 14KK30				
Che14KK29	F	W	14KK29				
Che14KK40	F	Cb1		14KK40			15KK62
Che14KK43	F	W		14KK43			
Che14KK45	F	Cb1			14KK45; 14KK48		
Che14KK46	F	W			14KK46		
Che14KK47	M	Ca			14KK47		
Che14KK49	M	Ca			14KK49		
Che14KK50	F	Cb1			14KK50		
Che14KK51	F	Ca			14KK51		
Che14KK52	F	W			14KK52		
Che14KK53	M	E			14KK53		
Che14KK54	M	Ca			14KK54		
Che14KK55	F	Ca			14KK55		
Che14KK56	M	Jc			14KK56		
Che14KK57	F	W			14KK57		
Che14KK58	F	Jc			14KK58		
Che14KK59	F	Jc			14KK59		
Che14KK60	F	W			14KK60		
Che15KK31	M	Cb1				15KK31	
Che15KK58	F	Jc					15KK58
Che15KK59	F	Jc					15KK59;15KK61
Che15KK60	F	AE					15KK60
Che15KK63	F	Cb1					15KK63;15KK64
Che15KK65	F	Ca					15KK65;15KK66
Che15KK67	M	Cb1					15KK67
Che14KK26	failed	Ca	not enough loci for indiv ID				

Table 7. Sex of Hector's dolphins sampled off Kaikoura, north (KK-N) and south (KK-S) of the Kaikoura Canyon, and the probability (p) of a deviation from a 1:1 sex ratio.

	2014			2015			2014-15 Total		
	KK-N	KK-S	Total	KK-N	KK-S	Total	KK-N	KK-S	Total
Samples	49	37	86	59	12	71	108	49	157
Individuals	43	30	73	42	9	51	80	37	117
Females	25	16	41	24	7	31	45	21	66
Males	17	14	31	18	2	20	34	16	50
Unknown	1		1				1		1
p	0.280	0.856	0.289	0.441	0.180	0.161	0.260	0.511	0.163

Table 8. Abundance (N_{1+}) of Hector's dolphins age 1^+ north (Kaikoura-North) and south (Kaikoura-South) of the Kaikoura Canyon, as well as within the combined study area encompassing both local populations, estimated by genotype recapture.

Study Area	Individuals Sampled			Annual Genotype Recaptures	N_{1+}	95% CL	CV
	Total	2014	2015				
Kaikoura-North	80	43	42	5	314	216 - 483	0.32
Kaikoura-South	37	30	9	2	102	68 - 175	0.40
Combined Kaikoura	117	73	51	7	480	342 - 703	0.29

Table 9. Pairwise F_{ST} values among Hector's dolphin populations in Cloudy Bay, Kaikoura-North and Kaikoura-South calculated from mtDNA (below diagonal; shaded gray) and microsatellites (above diagonal). All values were significant at $p < 0.001$*.

	n mtDNA	Cloudy Bay	Kaikoura-North	Kaikoura-South
n msats	-	147	80	37
Cloudy Bay	153	-	0.005*	0.012*
Kaikoura-North	80	0.016*	-	0.013*
Kaikoura-South	37	0.057*	0.081*	-

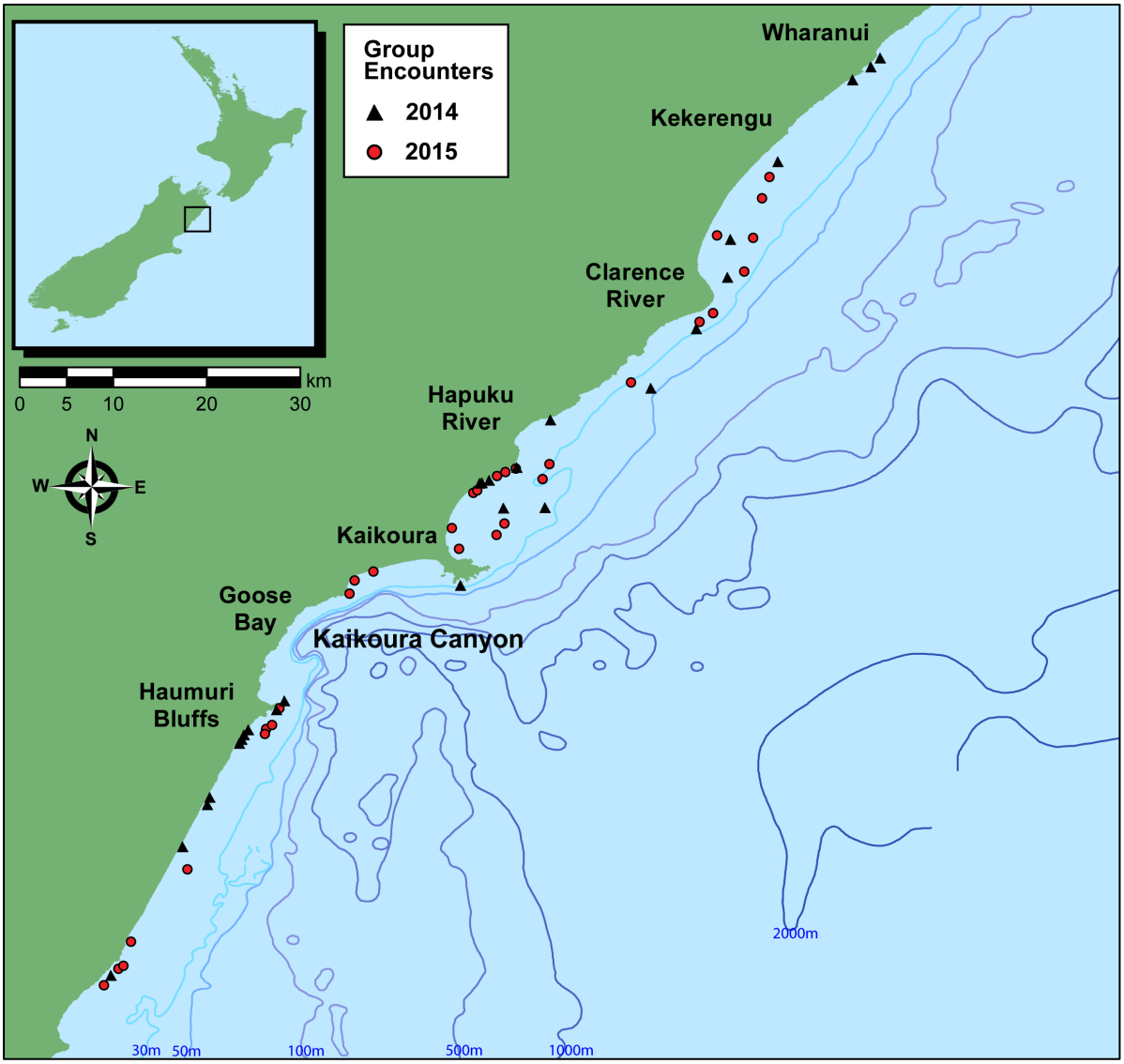

Figure 1. Hector's dolphin groups encountered in 2014 (30 groups) and 2015 (31 groups). Blue lines indicate water depth, as labeled, obtained from Land Information New Zealand (2012).

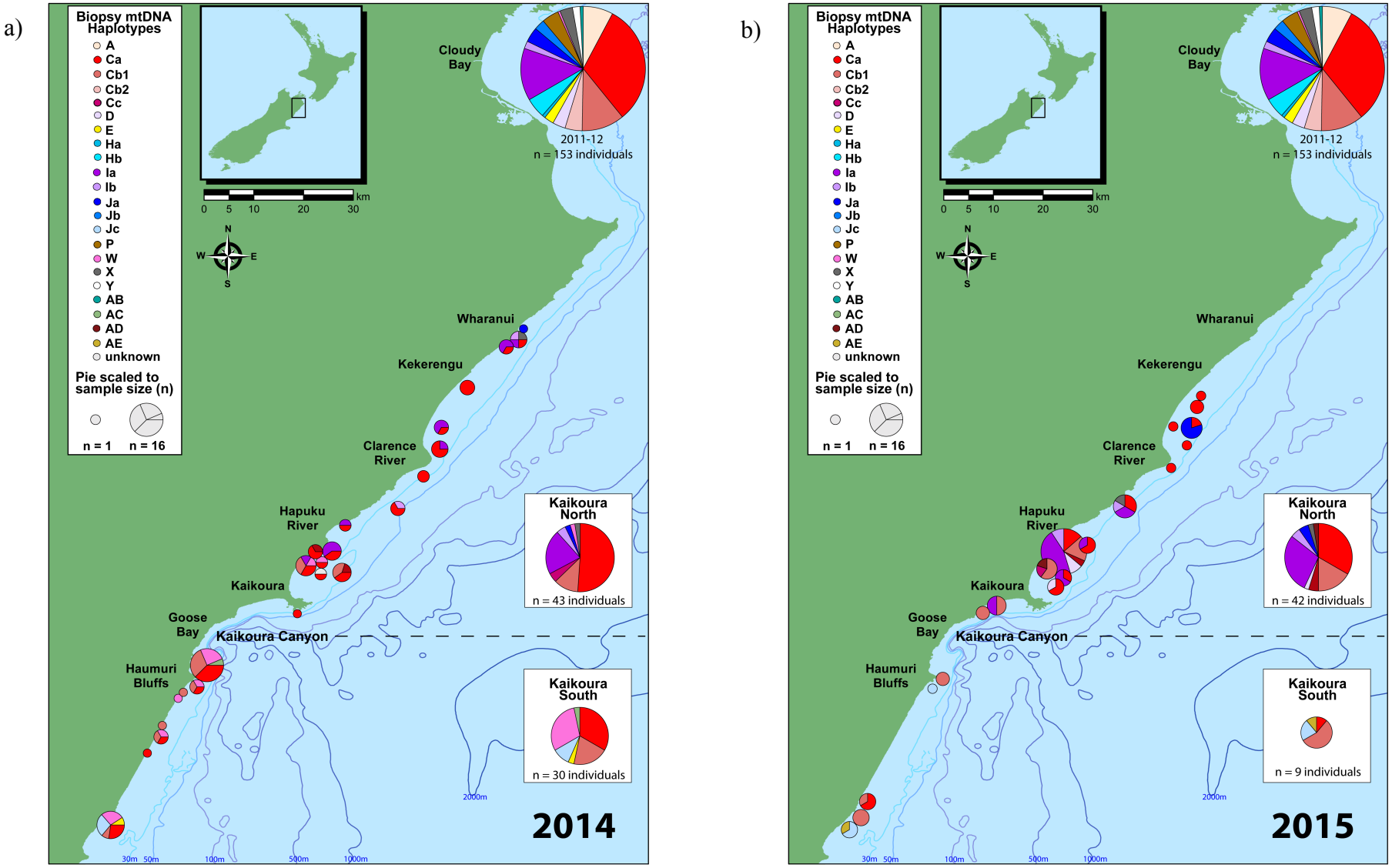

Figure 2. Hector's dolphin skin biopsy samples collected off Kaikoura in (a) 2014 and (b) 2015, as well as in Cloudy Bay in 2011-12 (Hamner *et al.* 2013) with shading to indicate mitochondrial (mt) DNA control region (576 bp) haplotypes.

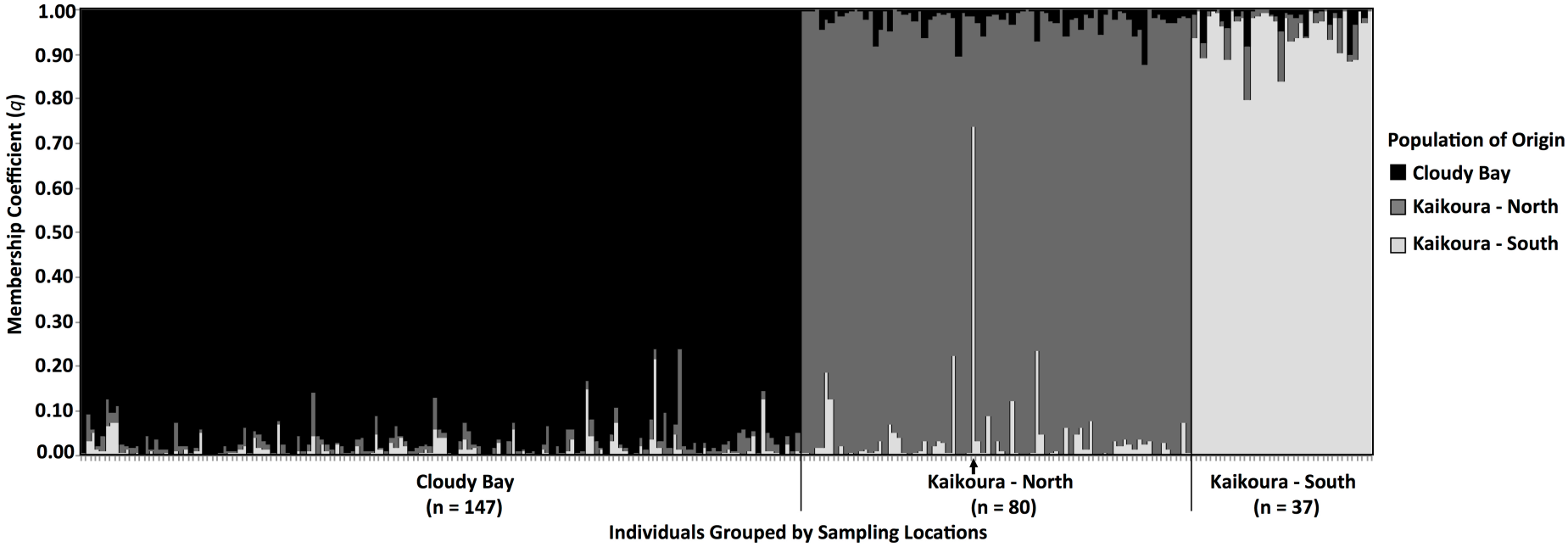

Figure 3. Assignment of Hector's dolphin individuals to Cloudy Bay, Kaikoura-North, or Kaikoura-South (K=3 populations) based on their microsatellite genotypes using the program *Structure* with 'UsePopInfo' option. Che14KK80, indicated by the arrow, is likely the offspring of a Kaikoura-North mother and immigrant father from Kaikoura-South.

Supplementary Material 1. Sex-specific genotype recapture abundance (N_{1+}) estimates for Hector's dolphins age 1^{+} north (Kaikoura-North) and south (Kaikoura-South) of the Kaikoura Canyon, as well as within the combined study area encompassing both local populations (Combined Kaikoura). No males were recaptured in Kaikoura-South, therefore, abundance could not be estimated for this group. *Includes one individual with unknown sex.

	Kaikoura-North			Kaikoura-South			Combined Kaikoura		
	Female	Male	Total	Female	Male	Total	Female	Male	Total
2014 individuals	25	17	43*	16	14	30	41	31	73*
2015 individuals	24	18	42	7	2	9	31	20	51
Annual recaptures	4	1	5	2	0	2	6	1	7
Total individuals	45	34	80*	21	16	37	66	50	117*
N (95% CL)	129 (90-201)	170 (97-326)	314 (216-483)	44 (31-74)		102 (68-175)	191 (139-281)	335 (185-650)	480 (342-703)
CV	0.33	0.52	0.32	0.37		0.4	0.29	0.53	0.29