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# Paternity assignment and demographic closure in the New Zealand southern right whale

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## Abstract

**The identification and characterization of reproductively isolated subpopulations or 'stocks' are essential for effective conservation and management decisions. This can be difficult in vagile marine species like marine mammals. We used paternity assignment and 'gametic recapture' to examine the reproductive autonomy of southern right whales (*Eubalaena australis*) on their New Zealand (NZ) calving grounds. We derived DNA profiles for 34 mother–calf pairs from skin biopsy samples, using sex-specific markers, 13 microsatellite loci and mtDNA haplotypes. We constructed DNA profiles for 314 adult males, representing 30% of the census male abundance of the NZ stock, previously estimated from genotypic mark-recapture modelling to be 1085 (95% CL 855, 1416). Under the hypothesis of demographic closure and the assumption of equal reproductive success among males, we predict: (i) the proportion of paternities assigned will reflect the proportion of the male population sampled and (ii) the gametic mark–recapture (GMR) estimate of male abundance will be equivalent to the census male estimate for the NZ stock. Consistent with these predictions, we found that the proportion of assigned paternities equalled the proportion of the census male population size sampled. Using the sample of males as the initial capture, and paternity assignment as the recapture, the GMR estimate of male abundance was 1001 (95% CL 542, 1469), similar to the male census estimate. These findings suggest that right whales returning to the NZ calving ground are reproductively autonomous on a generational timescale, as well as isolated by maternal fidelity on an evolutionary timescale, from others in the Indo-Pacific region.**

*Keywords*: gametic mark recapture, geneflow, population structure

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## Introduction

Defining population structure is critical for the effective management of species, particularly those that have undergone exploitation. However, characterization of population structure can be problematic in marine species with large effective population sizes, particularly when there are no obvious barriers to geneflow. Here we focus on the southern right whale (*Eubalaena australis*), which was subject to extensive commercial whaling in the nineteenth century and illegal Soviet whaling in the twentieth century (IWC 2001; Tormosov *et al*. 1998). The species was targeted on both its high-latitude,

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offshore summer feeding grounds and sheltered, coastal winter breeding or calving grounds (IWC 1986, 2001). Long-term photo-identification studies have shown female southern right whales exhibit fidelity to calving grounds, and return repeatedly to the same coastal sites to calve (Bannister 1990; Best 1990; Burnell 2001; Payne 1986). This fidelity acts as an isolating mechanism, creating matrilineal subpopulations, and contributes to the convention that the biological unit used to define southern right whale stocks or subpopulations is the calving ground (IWC 2001). This is consistent with genetic studies showing that southern right whale calving areas in southwest Australia (SWA), New Zealand (NZ), Argentina and South Africa are differentiated based on maternally inherited mitochondrial DNA (mtDNA) haplotype frequencies (Carroll *et al.* 2011b; Patenaude *et al.* 2007).

In the South Pacific, southern right whales have two major calving aggregations: one in NZ and one in SWA, approximately 2500 km apart (a third remnant calving ground is also found in southeast Australia; Burnell 2001; Carroll *et al.* 2011b; Kemper *et al.* 1997; Patenaude & Baker 2001). The NZ subpopulation comprises calving grounds in the NZ subantarctic Auckland Islands and around mainland NZ (North and South Islands) that represent a single matrilineal subpopulation (Carroll *et al.* 2011b). The SWA subpopulation comprises calving grounds across South and West Australia and was estimated to number 2900 whales in 2009, based on long-term aerial survey and photo-identification data (Bannister 2011; Burnell 2001). There is structuring of maternal lineages across NZ and Australia, with the NZ and SWA subpopulations showing significant genetic differentiation in mtDNA haplotype frequencies ($F_{ST} = 0.08$, $\Phi_{ST} = 0.16$, $P < 0.001$; Carroll *et al.* 2011b). The structuring follows the definition of subpopulation or stock proposed by Wade & Angliss (1997), whereby demographic processes operating within the subpopulation are more important than immigration from other subpopulations. However, the degree of reproductive isolation between the NZ and SWA calving grounds remains uncertain. There was a small but significant differentiation between the NZ and SWA calving grounds based on bi-parentally inherited microsatellite allele frequencies, suggesting recent divergence or some degree of isolation (13 loci; $F_{ST} = 0.004$, $G'_{ST} = 0.020$, $P < 0.05$; Carroll *et al.* 2011b).

However, genetic differentiation can be influenced more by a reduction in population size than by time since isolation (Hedrick 1999). Fluctuations in population size can cause changes in allele frequencies that are dependent on the severity and duration of the demographic bottleneck (Hedrick 1999; Luikart *et al.* 1998). Therefore, the extensive whaling of both the NZ and Australian stocks during the nineteenth and twentieth centuries and resulting demographic bottlenecks could have contributed to the observed level of differentiation (IWC 2001). Alternatively, the current degree of geneflow may be lower now than historically, under the assumption of density-dependent migration (Fowler 1984; Neubert & Caswell 2000).

The pattern of strong structuring of maternal lineages but weaker differentiation in microsatellite loci found in southern right whales suggests female philopatry but male geneflow. This is a common life history pattern found in mammals (Greenwood 1980), including other cetacean species such as sperm whales *Physeter macrocephalus* (Engelhaupt *et al.* 2009; Lyrholm *et al.* 1999), humpback whales *Megaptera novaeangliae* (Baker *et al.* 1998; Palumbi & Baker 1994), bottlenose dolphins *Tursiops* spp. (Möller & Beheregaray 2004) and Gray's spinner dolphins *Stenella longirostris longirostris* (Oremus *et al.* 2007). Various hypotheses have been proposed for sex-biased dispersal, including resource competition, inbreeding avoidance and local mate competition (Dobson 1982; Greenwood 1980; Perrin & Mazalov 2000; Pusey 1987).

A previous study used dispersal and assignment tests to examine the possibility of male-biased geneflow in the NZ southern right whale, using a large sample ($n = 605$) from the NZ subantarctic calving grounds (Carroll *et al.* 2011b). No significant pattern of sex-biased dispersal was found between the NZ and SWA stocks (Carroll *et al.* 2011b). Given the low level of genetic differentiation between these two proposed stocks, it was not surprising the Bayesian clustering program STRUCTURE (Pritchard *et al.* 2000) failed to differentiate between them. It is recognized that while assignment tests work better as populations become more differentiated ($F_{ST} \geq 0.10$), parentage analyses may be more suitable for testing dispersal among populations when there is low differentiation ($F_{ST} \leq 0.01$) (Goudet *et al.* 2002; Manel *et al.* 2005; Waser & Hadfield 2011). Paternity assignment also allows investigation of geneflow on an ecologically meaningful, or generational, timescale, whereas tests of differentiation examine differences between stocks on an evolutionary timescale (Christie 2010).

For example, Garrigue *et al.* (2004) used paternity assignment to test the hypothesis of reproductive autonomy in the New Caledonian humpback whales. A gametic mark–recapture (GMR) estimate of abundance was derived from paternity assignments to test for demographic closure of the population. The sample of adult males and calves was considered separate 'capture' occasions, and males were 'recaptured' if they were assigned as fathers, that is captured as gametes. This information was used in a simple mark–recapture model to calculate the number of reproductive males in

the New Caledonian humpback whale population. The resulting GMR estimate of male abundance was compared with the census estimate derived from a microsatellite genotype mark–recapture study. The close agreement of the gametic and census estimates suggests the population was reproductively and demographically closed. If there were a substantial number of males from other populations contributing to the paternity of the New Caledonian calves, then the GMR estimate would have been considerably larger than the census estimate (Garrigue *et al.* 2004). Importantly, this method has the potential to test the hypothesis of demographic closure using only a relatively small collection of samples from the population in question, which is beneficial because of the difficulty in thoroughly sampling highly mobile or migratory marine species.

Here we use GMR to investigate reproductive autonomy and estimate the male population size of the NZ southern right whale. Given the hypothesis of demographic closure, we predict that (i) the proportion of paternities assigned will reflect the proportion of males from the NZ stock sampled and (ii) the GMR estimate of male abundance will be consistent with the 2009 estimate of male census population size of 1085 (95% CL 855, 1417) derived from a genotypic mark–recapture (POPAN super-population) modelling (Carroll *et al.* 2011a). We extend the GMR method to explicitly test the assumption of equal reproductive success and assess our power to reject the null hypothesis of panmictic mating.

We used three methods to infer paternity: strict exclusion (Chakraborty *et al.* 1974), a maximum likelihood method (ML; as implemented by the program CERVUS v3.0; Kalinowski *et al.* 2007) and a Bayesian method (Christie 2010). Strict exclusion is a powerful tool for assigning paternity, but does not account for genotyping error, mutation or the possibility of multiple males being non-excluded as fathers. Both the ML and Bayesian methods can account for genotyping error and mutation. Simulations suggest the Bayesian and ML methods should perform equally well when the true number of fathers is close to the expected number of potential fathers (Christie 2010). In this study, the expected number of potential fathers should be equivalent to the estimate of adult male abundance for the NZ subantarctic. However, if the number of potential fathers is higher than expected, that is, there is a high level of interchange with SWA, the Bayesian method is expected to perform better (Christie 2010). This is the first time that both the Bayesian and ML methods have been tested empirically.

Here we use these parentage methods to resolve the degree of demographic closure in the endangered NZ southern right whale stock, with a focus on male geneflow. These analyses will help in improving conservation and management decisions for this marine species by assessing the degree of isolation of the NZ and neighbouring subpopulations. In doing so, we examine the comparative usefulness of various parentage analysis methods. Furthermore, it also provides information on the mating patterns of the southern right whale. While paternity has been studied in North Atlantic right whales *Eubalaena glacialis* (Frasier *et al.* 2007) and humpback whales (Cerchio *et al.* 2005), this is the first to investigate paternity in the southern right whale. The timing and location of southern right whale mating behaviour are currently unknown, so it is hard to assess the potential connectivity between populations. Evidence from long-term photo-identification and behavioural studies suggests that mating behaviour seen in other winter aggregations does not result in conception (Best *et al.* 2003; Payne 1986). Thus, when and where this species mates has been described an 'enigma' (Payne 1986) that this work goes some way to resolving.

## Methods

### *Sample collection*

Surveys were conducted from small vessels (4.6–5.2 m) at Port Ross, Auckland Islands (50° 32′S, 166° 15′E) during the austral winters of 1995–1998 and 2006–2009 as described by Patenaude *et al.* (2001) and Carroll *et al.* (2011c). Skin biopsy samples were collected using a small, stainless steel biopsy dart deployed from a crossbow in 1995–1998 (Lambertsen 1987), or a modified veterinary capture rifle in 2006–2009 (Krützen *et al.* 2002). Darts were sterilized in 70% ethanol and by flame sterilization between deployments. Sloughed skin samples were also collected during the 1998 field season using a sterile scouring pad attached to the end of a blunt arrow fired from a crossbow (Harlin *et al.* 1999). Around the North and South Islands of NZ (mainland NZ), biopsy samples were collected opportunistically by NZ Department of Conservation employees using a modified veterinary capture rifle (Krützen *et al.* 2002). Skin samples were preserved in 70% ethanol on location and transferred to the University of Auckland for storage at −20 °C.

A calf was identified as a whale that appeared to be less than half the length of the accompanying whale. An adult in close association with a calf was assumed to be its mother. Linked observations of mothers and calves, presumed to be mother and offspring, are referred to as mother–calf pairs. The sample codes and DNA profiles of calves, and associated mothers, were identified through field notes taken during the 2006–2009

field surveys. Mother–calf pairs were also identified from the mainland NZ data set using field notes that were provided by the NZ Department of Conservation employees who collected the samples.

All adult and calf males sampled during the 1995–1998 field expeditions and all adult males sampled during the 2006–2009 expeditions were considered candidate fathers. Male southern right whales reach reproductive maturity at 3–6 years of age (Whitehead & Payne 1981), and the species has a high annual survival rate (0.990, 95% CL 0.985, 0.996; Brandão *et al.* 2010) and longevity suggesting males that were sampled during the 1995–1998 expedition are likely to be mature and alive to father calves between 2006 and 2009. Only calves sampled during the 2006–2009 field seasons were considered for the paternity analysis.

### *DNA profiling*

Total genomic DNA was extracted from skin biopsy samples using standard proteinase K digestion and phenol/chloroform methods (Sambrook *et al.* 1989), as modified for small samples by Baker *et al.* (1994). Molecular identification of sex and sequencing of the mtDNA control region (500 bp) was conducted following methods previously described in detail by Carroll *et al.* (2011b). Samples were genotyped using 13 microsatellite loci (GT23; Bérubé *et al.* 2000; TR3G1, TR3G2, and TR3F4; Frasier *et al.* 2006; GATA28 and GATA98; Palsbøll *et al.* 1997; EV1, EV37 and EV14; Valsecchi & Amos 1996; RW18, RW31, RW410, and RW48; Waldick *et al.* 1999) as described in Carroll *et al.* (2011c). Only samples that amplified at a minimum of 11 or more loci were included in further analyses to improve the power of the paternity assignment.

### *Paternity assignment*

Paternity assignment was conducted using three different methods: strict exclusion, the maximum likelihood (ML) method implemented in programme CERVUS and the Bayesian method of Christie (2010). The strict exclusion method involves the comparison of the genotypes of mother-offspring or mother–calf pairs, which allows the maternal allele at each locus to be excluded. The paternal alleles in the calf are then identified, and the genotypes of all candidate males are then compared to this inferred paternal genotype. Paternity is assigned when only one male is non-excluded as the father (Jamieson & Taylor 1997).

The strict exclusion method is a powerful tool; however, it does not account for multiple non-excluded males (which can share alleles by chance) or situations where the true father could be excluded because of genotyping error or mutation. Thus, the ML method of Marshall *et al.* (1998) as revised by Kalinowski *et al.* (2007) and the Bayesian method of Christie (2010) were also used to assign paternity.

The ML method of Kalinowski *et al.* (2007), implemented in the program CERVUS, compares the likelihood of the two most likely fathers. For each calf, the difference between the likelihoods of the two most likely fathers produces a $\Delta$ score. Simulations were conducted to estimate the critical values of $\Delta$ required to assign paternity with a certain degree of confidence, based on the assumptions made about the population (e.g. population size). Paternities assigned at both the 95% and 80% confidence levels were reported, as determined by the critical $\Delta$ score. CERVUS additionally reports the probability of non-exclusion, which is the probability that an unrelated male will not be excluded as the likely father (Marshall *et al.* 1998). CERVUS was used to assign paternity of candidate males and the critical $\Delta$ score was estimated using 10 000 simulations, a genotyping error rate of 1%, and allowing for missing data at a maximum of two of 13 loci. The simulations were run under the assumption that the number of candidate males was 1085 based on genotypic mark–recapture modelling (Carroll *et al.* 2011a), and the proportion of candidate males sampled was calculated to be 30% (314/1085).

The method of Christie (2010) was used to assign paternity of calves to candidate fathers without maternal data. Briefly, this method involves identifying all putative parent–offspring pairs, and for each pair, estimating the unbiased exclusion probability ($Pr(\delta)$; Christie 2010. The expected number of false parent–offspring pairs is next calculated by combining this exclusion probability with the total number of pair-wise comparisons. This expected number of false assignments is used as a prior in a Bayesian framework to calculate the probability that a putative parent–offspring pair is false, given the frequencies of shared alleles ($Pr(\Phi \mid \lambda)$). A simulation method is employed that creates null data sets with the same number of samples and allele frequencies as the original data set. All father–offspring pairs found in the null data sets are false (i.e. share alleles by chance) and are used to create a distribution of an unbiased exclusion probability, $Pr(\delta)$, termed $Pr(\delta)_F$. The proportion of simulations with $Pr(\delta)_F < Pr(\delta)$ (i.e. simulated father–offspring pairs that share alleles less common than that of the father–offspring pair under consideration) is combined within a Bayesian framework to calculate the probability that the putative pair shares the observed alleles by chance, or $Pr(\Phi \mid \lambda)$.

The method of Christie (2010) was extended to account for genotyping error/mutation and missing data. Using R code (R Development Core Team 2011),

we identified all putative father–offspring pairs, while allowing for missing data and permitting one locus to mismatch. The $Pr(\Phi|\lambda)$ for those pairs with one mismatching locus was estimated by simulating 10 000 null data sets with the 12 matching loci.

$Pr(\Phi|\lambda)$ was calculated for each putative father–offspring pair, and those that had a $Pr(\Phi|\lambda) \leq 0.2$ were genotyped at an additional three loci as an independent check on the assignment. The putative father–offspring pairs were categorized into '95% confidence' assignments, those with $Pr(\Phi|\lambda) \leq 0.05$ and '80% confidence' assignments, those with $Pr(\Phi|\lambda) = 0.05$–$0.20$, to facilitate comparison with the CERVUS results.

### *Augmenting DNA profiles*

Simulations suggest up to 44% of true fathers are excluded when using the conservative 95% confidence ML analysis (see Cerchio *et al.* 2005; under conditions described by; Marshall *et al.* 1998). This would bias downward the number of gametic recaptures and subsequently produce a large positive bias for the GMR estimate of male abundance. Accordingly, we took a relatively relaxed initial approach to identifying father–offspring pairs, by considering all paternities assigned with a minimum of 80% confidence from the ML and Bayesian methods.

To increase confidence in the individual paternity assignments, all putative parent-offspring tryads were genotyped at an additional three loci. These additional loci were GT211 (Bérubé *et al.* 2000), TR3G10 (Frasier *et al.* 2006) and RW34 (Waldick *et al.* 1999), and methods for genotyping using these loci are described in Table S1, Supporting Information. The probability of non-exclusion was recalculated in CERVUS for the paternity assignments using the subset of samples amplified for these loci. Additionally, the Mendelian agreement of the mother, calf and father genotypes was used as a check on the assignment, such that if the tryads did not agree at these three loci, the assignment was considered false.

### *GMR abundance estimate*

A two-sample Chapman's modified Lincoln Peterson model (Amstrup *et al.* 2005) was used to estimate the number of reproductive males ($N$) through GMR in the NZ population (eqn 1), and compared with the census estimate of male abundance from genotypic mark–recapture modelling (Carroll *et al.* 2011a). The first capture occasion, $n_1$, was considered to be the adult males sampled around the NZ (both mainland NZ and NZ subantarctic) over the period 1995–2009. The second capture occasion, $n_2$, was the sampling of calves. The recapture, $m$, was considered to be assignment as a father (gametic recapture).

$$N = \frac{(n_1 + 1)(n_2 + 1)}{m + 1} \quad \text{(eqn1)}$$

### *Assessing the power of GMR*

To test the power of our analyses to reject the null hypothesis of panmictic mating between NZ and SWA, we undertook two analyses. The first analysis used CERVUS and allele frequencies from both the NZ and SWA populations to simulate the number of paternities that would be assigned to 34 calves, under the following assumptions: the number of candidate males was 2500 (of which 13% were sampled), and candidate males were typed at a minimum of 11 loci with a 1% error rate. The value of 2500 candidate males was approximated from the census estimate of the male population size of 1085 for NZ and half the overall abundance estimate for the SWA subpopulation of 2900 whales in 2009 (Bannister 2011; Carroll *et al.* 2011a).

We ran the simulation 1000 times and counted the number of simulations where the number of paternities assigned with >80% confidence was equal or greater to the number of paternities assigned in the actual analysis. The number of times this occurred divided by the total number of simulations determined the $P$-value.

In addition, we used R to simulate a randomly mating population of right whales comprising 1085 NZ males and 1450 SWA males. From this combined population, 34 calves were assigned a father under the assumption of equal reproductive success amongst candidate males. Next, we took a sample of NZ males from the simulated population of the same size as the actual number of males sampled from the NZ population ($n = 314$). The number of fathers in the sample was then tallied, and the simulation procedure repeated 10 000 times. The $P$-value was calculated as the number of simulations where there were 10 or more fathers in the sample, divided by the total number of simulations.

### *Testing the assumption of equal reproductive success*

The GMR method relies on the assumption of equal reproductive success between males. If there is a skew in male reproductive success, with fewer males than expected under random mating fathering calves, then this would decrease the number of gametic recaptures identified. This would create a positive bias in the GMR estimate of male abundance. To test the assumption of equal reproductive success, we used DADSHARE

(Available from http://www.zoo.cam.ac.uk/zoostaff/meg/software/DadShare.pdf; see Hoffman *et al.* 2003) to estimate the number of fathers that sired the calves that were not assigned paternities to the available candidate males. DADSHARE uses the maternal genotypes to identify paternal alleles in the offspring. The program then uses paternal alleles to calculate relatedness between all offspring with the method of Queller & Goodnight (1989). A clustering algorithm then constructs a dendogram that links the most closely related individuals. In addition, it uses a Monte Carlo simulation approach to generate average relatedness value (*r*-value) of the branch tips (i.e. putative siblings) based on randomization of the data set, and assuming a range of number of fathers for the offspring. The program simulated the average *r*-value of putative siblings based on one male fathering all offspring, and 2–6, 8 or 12 males each siring the same number of offspring, in addition to each offspring having a unique father. The mean *r*-value for each of these simulations is subsequently compared with the observed *r*-value of the putative siblings to interpret the significance of the results. As a check on this result, we used the program ML-relate that calculates maximum likelihood estimates of relatedness (Kalinowski *et al.* 2006). ML-relate tests the likelihood of two *a priori* hypotheses on the relatedness of two individuals. In this case, the hypothesis that the two calves identified by the DADSHARE analysis are half-siblings, against the alternate hypothesis that the two whales are unrelated. This is calculated by simulating genotypes using the allele frequencies in the sample, and examining the proportion of simulations where the two individuals have the putative relationship (Kalinowski *et al.* 2006).

By combining the paternity and DADSHARE analyses, we were able to estimate the observed distribution of male reproductive success, based on the 34 mother–calf pairs available. To test whether the observed distribution was different from that expected under the assumption of equal reproductive success, we conducted a randomization procedure to generate an expected distribution similar to that described by Frasier *et al.* (2007). Parameters of the randomization procedure included the number of adult males in the population, estimated from genotypic mark–recapture modelling, and the number of calves included in the paternity analysis. Males had an equal chance of being selected and were sampled with replacement until fathers were assigned to all 34 calves. After 1000 simulations, the mean and standard deviation (SD) of the number of males assigned zero, one, two or three offspring were calculated. A chi-squared test was then used to test for significant differences between the observed and expected distributions.

## Results

### *Sample collection and DNA profiling*

A total of 1188 samples were collected from southern right whales at the NZ subantarctic Auckland Islands during the winter field expeditions in 1995–1998 and 2006–2009. Given some variation in the quality and quantity of DNA, not all samples were genotyped at all 13 loci. DNA profiles (mtDNA haplotype, genetic sex identification and multilocus genotype of ≥9 loci) were constructed for 1089 samples, and matching of genotypes between and within years resolved 763 unique individuals (adults and calves) sampled over this 8-year period. Genotyping results for the 1995–1998 field seasons can be found in more detail in Carroll *et al.* (2011c). A total of 60 samples were also collected from southern right whales around mainland NZ between 2003 and 2009. DNA profiles were constructed for 59 samples, and the comparison between- and within years showed that there were 46 unique individuals sampled over the six-year period: 24 females, 21 males and one of unknown sex. Genotyping results for mainland NZ samples were previously published in Carroll *et al.* (2011b).

DNA profiles were available for a total of 315 adult, candidate males from the NZ subantarctic data set and 17 from the mainland data set. Any profile that did not amplify at a minimum of 11 loci was excluded, leaving DNA profiles for 299 candidate males from the NZ subantarctic data set and 15 males from the mainland NZ data set. This total represented 30% of the 1085 males estimated to be in the NZ stock (Carroll *et al.* 2011a). From field notes, we identified 32 mother–calf pairs collected from the Auckland Islands and four from mainland NZ (Fig. 1). However, two putative mothers were excluded as mothers of the associated calves, as the genotypes were clearly of unrelated individuals (mismatch at 6 of 13 loci). Inspection of the field notes revealed that there were multiple mother–calf pairs in the area when these samples were collected from the Auckland Islands. These two false mother–calf pairs were excluded, leaving 34 mother–calf pairs for analysis.

Only those samples that were successfully genotyped at between 11 and 13 loci were included in the paternity analyses, and the least polymorphic 11 loci gave a conservatively estimated probability of identity of 7.8E-14 (Paetkau *et al.* 1995). As there are likely to be relatives in the data set, we calculated the conservative probability of identity of siblings for the least variable 11 loci; 1.7E-05 (Waits *et al.* 2001). Overall, samples included in the analyses (mother–calf pairs and candidate males) were amplified at an average of 12.5 microsatellite loci

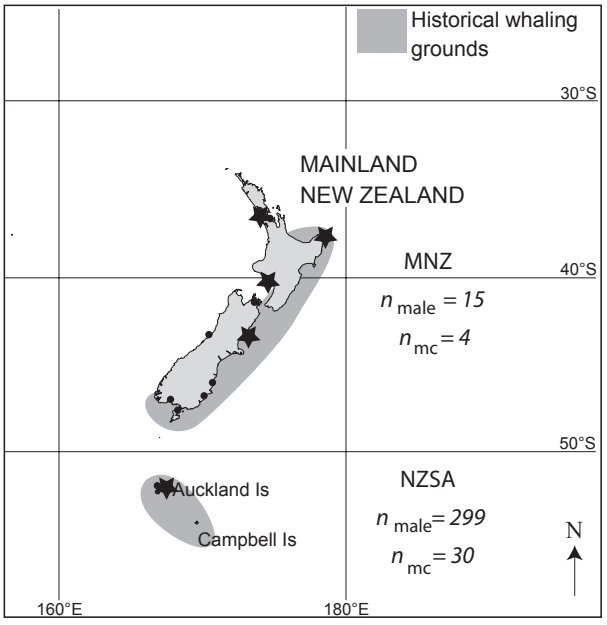


**Fig. 1** Sampling locations and sample size of candidate males (black dots; $n_{male}$) and mother–calf pairs (black stars; $n_{cc}$) from the New Zealand subantarctic (NZSA) and mainland New Zealand (MNZ) southern right whale calving grounds. Historical winter range of southern right whale shown by shading (Dawbin 1986; Jackson *et al.* 2011).

and each locus had, on average, 3.6% missing data. The per allele error rate for the data set was previously estimated at 0.65% (Carroll *et al.* 2011b).

### *Initial paternity assignment*

Using strict exclusion, eight of 34 (24%) calves were assigned paternities from among the 314 candidate males. The probability of non-exclusion for these assignments was between 7.21E-10 and 1.37E-05. Using the ML method with a 1% error rate, 10 of 34 (30%) calves were assigned paternities; eight with 95% confidence and two with 80% confidence. Using the Bayesian method, 10 paternities were assigned to nine calves with 95% confidence, and a further six paternities were assigned to five calves with 80% confidence (Tables 1 and 2). After reconciling paternity assignments made by more than one method, a total of 12 calves were assigned a single father by two or more methods. Three additional calves were assigned to multiple fathers, but this was resolved with data augmentation (see below). Of these 18 paternities, 11 were made with 95% confidence and seven made with 80% confidence by ML and/or Bayesian methods (Table 1).

In general, the three methods produced congruent results, with the same five candidate males assigned by all three methods and the same 10 candidate males assigned by any two methods. The main difference between the ML and Bayesian methods was that the ML method excluded maternal alleles, which limited the number of candidate fathers for each offspring and likely resulted in increased accuracy. Consequently, three calves were assigned two non-excluded fathers using the Bayesian method. One calf was assigned one father with 95% confidence and one with 80% confidence (Table 2). In two of the three cases of multiple paternities, the ML and Bayesian method agreed on the same two males as the most likely fathers.

**Table 1** Number of paternity assignments to calves from the NZ southern right whale calving ground using strict exclusion, the maximum likelihood method of Kalinowski *et al.* (2007) implemented in CERVUS with 1% error rate (ML – 1% error) and the Bayesian method of Christie (2010). The latter two methods are further categorized into assignments made with 80% and 95% confidence. The number of calves assigned multiple paternities are also noted for Bayesian method (ML method does not allow for multiple paternities). The number in parentheses represents those assignments retained after further validation (see Methods)

| | N paternities assigned | | |
|---|---|---|---|
| Method | 95% confidence | 80% confidence | Multiple paternities |
| Strict exclusion | 8 (7) | | – |
| ML – 1% error | 8 (8) | 2 (2) | – |
| Bayesian | 10 (7) | 6 (1) | 3 (0) |
| All methods | 11 (8) | 7 (2) | 3 (0) |

### *Confirmation of assignments with additional loci*

We confirmed initial paternity assignments by genotyping all putative offspring-parent tryads at three additional loci and employing simple Mendelian incompatibility. In the three cases of multiple non-excluded fathers, this method resolved the ambiguity. Of the remaining 12 calves assigned to a single father, seven were assigned with 95% confidence and five were assigned with 80% confidence. Genotyping at three additional loci excluded one of seven paternities made with 95% confidence and four of five paternities assigned with 80% confidence, and these were not used in further analysis. The DNA sample from one male assigned as a father (Eau06AI111) did not have sufficient DNA to amplify the additional loci. As this was male assigned as a father with 95% confidence by both ML and Bayesian methods, the assignment was retained. In total, 10 of 15 paternities were validated using additional loci and were retained for further analysis (Table S2, Supporting Information).

**Table 2** Details of all paternity assignments made to calves from the NZ southern right whale calving ground (one parent known). For each putative assignment, the following are listed: mtDNA haplotype (mtDNA) of calf, father and mother; sex of calf (sex); number of loci at which the pair match ($n_{loci}$); probability of non-exclusion for the assignment ($P_{NE}$); whether the assignment was made using strict exclusion (ST); confidence level of match calculated using maximum likelihood method of Kalinowski *et al.* (2007) (ML %) and Bayesian method of Christie (2010) (Bayesian Pr($\Phi|\lambda$)). As a check on the assignments samples were genotyped at an additional three loci and the number at which putative pairs matched are listed (3loci) and whether there was agreement with maternal data (maternal data) is described. Shaded rows indicate those assignments that were retained for the gametic mark–recapture estimate. The number of loci out of the additional three amplified the father–offspring pair match at (3 loci) and the revised probability of non-exclusion for the assignment including these three loci, with one parent known ($P_{NE}$ (1))

| Calf | | Mother | Father | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| mtDNA | Sex | mtDNA | mtDNA | $n_{loci}$ | $P_{NE}$ | ST | ML (%) | Bayesian Pr ($\Phi|\lambda$) | 3 loci | $P_{NE}$ (1) | Maternal data |
| BAKHAPB′[1] | M | BAKHAPB′ | BAKHAPB+ | 12 | 6.50E-08 | N | <80 | 0.04 | 1 | - | Y |
| BAKHAPB′[1] | M | BAKHAPB′ | BAKHAPA | 12 | 6.50E-08 | Y | 95 | 0.02 | 3 | 2.17E-08 | Y |
| BAKHAPA [2] | F | BAKHAPA | BAKHAPA | 11 | 2.56E-08 | N | 80 | 0.04 | 3 | 1.08E-11 | Y |
| BAKHAPA [2] | F | BAKHAPA | BAKHAPB+ | 12 | 2.56E-08 | Y | <80 | 0.14 | 1 | - | N |
| BAKHAPD | M | BAKHAPD | BAKHAPA | 13 | 2.34E-08 | Y | 95 | 0.002 | 3 | 4.07E-10 | Y |
| BAKHAPD | F | BAKHAPD | BAKHAPA | 11 | 1.37E-05 | Y | <80 | 0.14 | 3 | - | N |
| BAKHAPB+ | M | BAKHAPB+ | BAKHAPA | 13 | 2.10E-06 | Y | 95 | >0.20 | 3 | 5.62E-11 | Y |
| BAKHAPA | F | BAKHAPA | BAKHAPA | 13 | 4.97E-09 | Y | 95 | <0.001 | 3 | 6.14E-12 | Y |
| BAKHAPB′ | F | BAKHAPB′ | BAKHAPB+ | 11 | 3.46E-07 | N | 80 | >0.20 | 3 | 5.77E-10 | Y |
| BAKHAPD | F | BAKHAPD | BAKHAPB+ | 13 | 7.36E-09 | Y | 95 | <0.001 | 3 | 1.14E-13 | Y |
| BAKHAPA | F | BAKHAPA | BAKHAPC | 12 | 8.00E-08 | N | 80 | 0.001 | 3 | 2.47E-09 | Y |
| BAKHAPB′ | M | BAKHAPB′ | BAKHAPB+ | 12 | 1.32E-07 | N | <80 | <0.001 | 0 | - | N |
| BAKHAPA [3] | F | BAKHAPA | BAKHAPA | 12 | 7.32E-07 | N | <80 | 0.15 | 0 | - | N |
| BAKHAPA [3] | F | BAKHAPA | BAKHAPB+ | 12 | 7.32E-07 | Y | 95 | 0.02 | 3 | 4.13E-08 | Y |
| BAKHAPD | M | BAKHAPD | BAKHAPD | 12 | 7.32E-07 | N | <80 | 0.18 | 1 | - | N |
| BAKHAPB+ | F | BAKHAPB+ | BAKHAPD | 12 | 7.21E-10 | N | <80 | 0.18 | 1 | - | N |
| BAKHAPB+ | M | BAKHAPB+ | BAKHAPD | 12 | 7.21E-10 | N | 95 | 0.005 | - | 2.81E-13 | Y |
| BAKHAPA | N/A | BAKHAPA | BAKHAPA | 12 | 4.90E-06 | N | <80 | 0.18 | 1 | - | N |

[1,2,3]Indicate the three multiple paternities.
[†]One locus failed to amplify in the calf.
[*]Not enough DNA to amplify additional loci for this sample.

### *GMR estimate of abundance*

There were $n_2 = 34$ calves and $m = 10$ paternity assignments providing a GMR estimate of 1001 males (95% CL 542, 1460). This is very similar to the estimate of male abundance estimated using genotypic mark–recapture and the POPAN super-population model of 1085 males (95% CL 855, 1417).

### *Assessing the power of GMR*

Under the assumption of panmictic mating between NZ and SWA with a combined pool of 2500 candidate males, CERVUS simulations predicted it was highly unlikely that 10 or more paternities would be assigned with >80% confidence given 314 of 2500 candidate males sampled and 34 calves ($P = 0.005$). Consistent results were found using the R-based simulations, which indicated there was a very small chance that 10 fathers would be in our sample of 314 under the assumption of panmictic mating ($P = 0.0057$).

### *Testing the assumption of equal reproductive success*

The randomization procedure conducted by DADSHARE suggested that, given the available dataset, the mean *r*-value between half siblings in the population was 0.268 (SD 0.02), assuming each offspring had a unique father. This is close to the 0.25 predicted by population genetics theory (Queller & Goodnight 1989). The clustering algorithm employed by DADSHARE showed there were six likely pairs of half siblings among the 34 calves (*r*-value = 0.26–0.49). The ML-relate analyses strongly supported the half-sibling relationship for four of six pairs of half-siblings ($P < 0.05$). Overall, the combined paternity and DADSHARE analysis with the ML-relate check showed 1057 males did not father any of the 34 calves, 26 fathered one calf, four fathered two calves and none fathered three calves (Fig. 2).

Under the assumption of equal reproductive success, the simulation procedure suggested that 1052 (SD 0.75) males would not father a calf, 33 (SD 1.48) would father

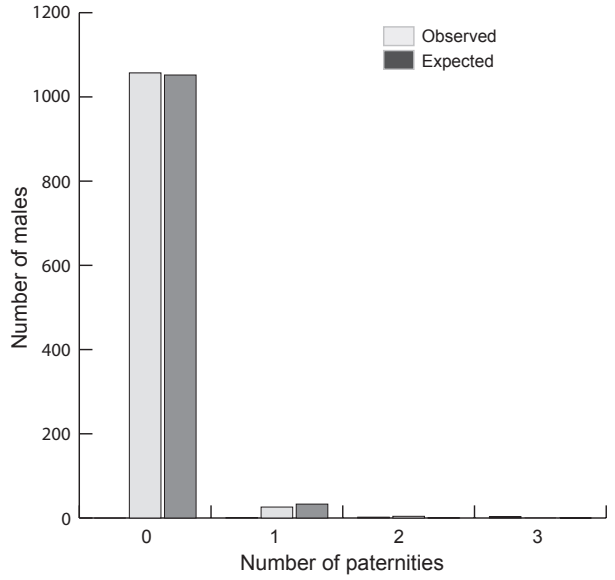


**Fig. 2** The number of single or multiple paternities assigned to males in the New Zealand southern right whale population, based on the paternity assignment of 34 calves. The observed distribution of reproductive success was determined using a combination of paternity assignment methods, DADSHARE and ML-relate analyses, and the expected distribution under the assumption of random mating was derived from simulation procedures in program R.

one calf, 0.53 (SD 0.73) would father two calves and 0.007 (SD 0.08) would father three calves (Fig. 2). A chi-square test showed there was not a significant difference between the observed distribution of reproductive success and the distribution expected under random mating ($P = 0.21$).

## Discussion

### *Paternity assignment and GMR estimate*

Here we present the first paternity analyses conducted for southern right whales, using DNA profiles from 34 mother–calf pairs and over 300 candidate fathers from the NZ stock. We further tested the hypothesis of 'demographic closure' by comparing the proportion of identified fathers with the expectations from the genotype mark–recapture modelling of male census abundance. The estimated census abundance of males in the NZ stock was 1085 in 2009 (95% CL 855, 1416), and 314 or 30% were sampled. The assignment of paternity to 10 of 34 calves represents 30% of the total. The agreement between the proportion of males sampled and paternities assigned is in close accordance with the assumption of demographic closure. Although each estimate will have its own bias and uncertainty (Palsbøll *et al.* 2005), the GMR estimate of 1001 males (95% CL 542, 1460) is consistent with the estimate of male abundance produced from genotypic mark–recapture modelling (1085 males, 95% CL 855, 1416; Carroll *et al.* 2011a). These findings are consistent with the hypothesis that the NZ stock is currently reproductively autonomous, supporting previous work showing that the NZ stock is currently isolated from its larger neighbouring stock, SWA (Carroll *et al.* 2011b).

The alternate hypothesis is that paternity is the result of panmictic mating among a much larger population. We tested our power to reject this alternate hypothesis by simulating the expected number of paternity assignments assuming a larger pool of candidate males. The SWA calving ground is the nearest substantial stock of southern right whales and was estimated to number 2900 whales in 2009 (no confidence limits or sex-specific estimates available; Bannister 2011). If males from both the SWA and NZ subpopulations were contributing to the NZ population, and assuming equal reproductive success amongst males, our simulations suggested the chance of assigning 10 paternities was ≤1%. In turn, this would have produced a much higher GMR estimate of male abundance. Therefore, it is unlikely we were drawing from a pool of candidate males substantially larger than the NZ subpopulation.

This work highlights the usefulness of paternity assignment and GMR in assessing the current reproductive autonomy of a population compared with traditional indices of genetic differentiation. Whaling drastically altered the distribution of southern right whales across NZ and Australia, and caused a worldwide demographic bottleneck in the species. The low degree of genetic differentiation between NZ and SWA may be a remnant of the historically larger populations, which under the assumption of density-dependent migration could have had a higher degree of geneflow. The results presented here suggest that the NZ subpopulation is currently demographically closed to substantial geneflow from SWA. As the NZ and SWA populations continue to increase in number, the restricted geneflow could begin to increase. It could be possible to monitor the change over time using the GMR approach, although it would require investment in continued sampling of the population in general, and mother–calf pairs in particular, over time.

The definition of stock used here does not preclude low levels of migration and geneflow, such as that documented by photo-identification studies between the NZ subantarctic and Head of the Bight (SWA) calving areas (Pirzl *et al.* 2009). However, the finding of demographic closure through reproductive autonomy suggests the degree of geneflow is low compared

with the number of whales that show fidelity to the NZ stock.

### *Confidence in GMR*

Here we used multiple methods and initially relaxed (80%) confidence levels to assign paternity to identify as many true fathers as possible. Further confidence in paternities was provided with genotyping putative father–offspring pairs at three additional loci; this excluded nearly half the relaxed putative assignments. Using this method to identify as many potential paternities as possible and then using Mendelian incompatibility at additional loci to confirm, paternity was chosen to provide information on paternities at a population level. Even though the data set has a low error rate, the large number of loci used and the chance of mutation mean that discrepancies between the genotypes of the father and offspring could occur implying the relaxed approach was appropriate. As the sample size of calves was small compared with other studies of baleen whales (e.g. Frasier *et al.* 2007), and the gametic recapture method is most sensitive to the number of assignments made, this approach helped ensure all potential fathers were identified. However, the implementation of additional, *post hoc* checks on the assignments provides confidence in the results.

In addition, the GMR method relied on the assumption that males have equivalent reproductive success. If there is an undetected skew in male reproductive success, with fewer males than expected under random mating fathering calves, then this would decrease the number of gametic recaptures identified. This would in turn result in a larger and less precise estimate of the number of reproductive males. As the expected number of paternity assignments was made given the expected proportion of males in the NZ population sampled, the bias does not appear to be significant. In addition, our results suggested that there was not a significant difference between the observed distribution of male reproductive success and the distribution expected under random mating.

### *Comparison of strict exclusion, ML and Bayesian methods*

The Bayesian method used here has been shown to be more accurate than the ML method implemented in CERVUS at assigning paternities when the number of potential fathers is large (Christie 2010). The more recent Bayesian method has not been used extensively, while the ML method is now commonly used in paternity assignment studies. For example, the ML method has been used in several publications on cetaceans to examine male reproductive success and reproductive autonomy (e.g. Frasier *et al.* 2007; Garrigue *et al.* 2004; Krützen *et al.* 2004). Here, we present the first time these two methods have been empirically tested on the same dataset.

The Bayesian method of Christie (2010) was modified to allow for missing data and one mismatching locus to allow for genotyping error and mutation. The Bayesian and ML methods showed agreement in seven of 10 paternities that were further validated. There were more assignments made with 80% confidence using the Bayesian method than the ML method, although these values may not be entirely equivalent. Nevertheless, all the putative fathers identified by the Bayesian method were also identified by CERVUS as either the most likely or next most likely father, but the Δ score did not reach the critical 80% significance value. The ML method is more powerful when analyses are conducted with maternal data (Kalinowski *et al.* 2007; Slate *et al.* 2000). When we repeated the paternity analyses without maternal data, approximately half the assignments declined in confidence and the probability of non-exclusion also decreased several fold. Allowing for the incorporation of maternal data into the Bayesian method and the use of additional loci would help to resolve this problem in future.

### *Southern right whale mating system*

The purpose of this paternity analysis and GMR was to evaluate the hypothesis of reproductive closure on a generational timescale. It was not intended to evaluate male reproductive success or male effective population size. However, these results contribute to the findings by others regarding these parameters in baleen whales.

Although paternity has not been investigated previously in southern right whales, it has been studied in the closely related North Atlantic right whale. Right whale mating behaviour involves surface active groups (SAGs), where a receptive female is the focus of courtship displays (Kraus & Hatch 2001). Male antagonistic behaviour involves stereotyped displays including body movements and 'gunshot' calls (Parks *et al.* 2005). Additionally, the physiology of right whales suggests the species displays one of the most extreme examples of sperm competition in mammals (Brownell & Ralls 1986). This mating system resulted in a skew in the reproductive success of male North Atlantic right whales, with a significant excess of males not being assigned any paternities and a greater number of males fathering multiple calves than expected under random mating (Frasier *et al.* 2007). Such a skew has also been documented in humpback whales (Cerchio *et al.* 2005; Nielsen *et al.* 2001), but in general the effect is much

smaller than terrestrial mammals (Frasier *et al.* 2007). This likely reflects the degree to which males can control access to mates in the marine versus terrestrial environments (Clapham 1996; Frasier *et al.* 2007).

In this study, the observed distribution of male reproductive success was not different from equal reproductive success. However, the number of calves available for analysis was small (34 mother–calf pairs) compared with the above-mentioned studies species (e.g. 127 humpback whale cow-calf pairs; Cerchio *et al.* 2005; 87 North Atlantic right whale cow-calf pairs; Frasier *et al.* 2007), and further research should be encouraged with larger sample sizes in future.

Furthermore, it is unclear exactly when and where southern right whales mate (Best *et al.* 2003; Payne 1986), highlighting the difficulty in determining the level of connectivity between recovering stocks. The results of this paternity analysis goes some way to answer this and suggests that whales that use the same calving ground are mating together. This is consistent with the observations of mating behaviours at the Auckland Islands (Patenaude *et al.* 1998) and the estimated gestation period of 11–13 months in the southern right whale (Lockyer 1984). However, it does contrast with the finding that SAGs in the South African and Argentinean waters focussed on primiparous or juvenile females, and thus were not likely to result in conception (Best *et al.* 2003; Payne 1986). The difference could be explained by contrasting patterns of habitat use between the two areas; all demographic classes are found at the NZ subantarctic whereas in South Africa, there are distinct nursery areas and areas frequented by whales without calves (Best *et al.* 2003; Patenaude 2002; Patenaude *et al.* 1998).

## Conclusion

This work shows that paternity analysis and GMR are useful tools for inferring demographic or reproductive closure when traditional population assignment tests are not sufficient. As such, they provide useful information towards defining management units for highly mobile marine species. In addition, it appears that while maternal fidelity may isolate southern right whale calving ground on an evolutionary timescale (mtDNA differentiation), male fidelity to calving grounds acts as an isolating mechanism on generational timescale (demographic closure).

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## Data accessibility

The R code for the Bayesian analysis is available at https://sites.google.com/site/parentagemethods/. The CERVUS input files and R code required for simulations are archived on Dryad (doi:10.5061/dryad.n630t).

## Supporting information

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

**Table S1** Three microsatellite loci used to augment the microsatellite genotype of southern right whales.

**Table S2** DNA profiles of parent-offspring tryads: mtDNA control region haplotype (500 bp; mtDNA), genetically identified sex and microsatellite genotype.

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