Human-mediated extirpation of the unique Chatham Islands sea lion and implications for the conservation management of remaining New Zealand sea lion populations

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Abstract

While terrestrial megafaunal extinctions have been well characterized worldwide, our understanding of declines in marine megafauna remains limited. Here, we use ancient DNA analyses of prehistoric (<1450–1650 AD) sea lion specimens from New Zealand's isolated Chatham Islands to assess the demographic impacts of human settlement. These data suggest there was a large population of sea lions, unique to the Chatham Islands, at the time of Polynesian settlement. This distinct mitochondrial lineage became rapidly extinct within 200 years due to overhunting, paralleling the extirpation of a similarly large endemic mainland population. Whole mitogenomic analyses confirm substantial intraspecific diversity among prehistoric lineages. Demographic models suggest that even low harvest rates would likely have driven rapid extinction of these lineages. This study indicates that surviving *Phocarctos* populations are remnants of a once diverse and widespread sea lion assemblage, highlighting dramatic human impacts on endemic marine biodiversity. Our findings also suggest that *Phocarctos* bycatch in commercial fisheries may contribute to the ongoing population decline.

Keywords: ancient DNA, Chatham Islands, extinction, fisheries bycatch, human impact, New Zealand sea lion, *Phocarctos hookeri*

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Introduction

Late Quaternary terrestrial megafaunal extinctions have been well studied worldwide, whereas marine megafaunal declines remain relatively poorly characterized. In addition, the causes of such biological impacts are often contentious, with both 'anthropogenic overkill' and 'climate driven extinction' hypotheses receiving broad support (Stuart 2014). Despite the relatively limited understanding of prehistoric impacts on coastal assemblages, a growing body of ancient DNA (aDNA) evidence (Boessenkool *et al.* 2009; De Bruyn *et al.* 2009; Rawlence *et al.* 2015a,b) suggests that many large, distinctive marine vertebrate species have, like their terrestrial counterparts, experienced major declines and extirpations.

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Isolated Pacific islands were colonized by humans only in recent millennia and provide valuable opportunities to assess anthropogenic impacts on endemic coastal biotas. The New Zealand (NZ) region in particular provides a unique system to assess such human impacts, as it has a well-preserved archaeological record (Worthy & Holdaway 2002). As the world's last major land mass to be colonized by humans (around 700 years ago) (Wilmshurst *et al.* 2008; Wilmshusrst *et al.* 2011), NZ presents a particularly strong system for assessing recent human impacts that are decoupled from major (e.g. glacial–interglacial) climatic shifts (Wanner *et al.* 2008).

Although *Phocarctos* sea lions are now restricted to southern NZ and its subantarctic islands, their remains are abundant in NZ's early prehistoric record, indicating a broad geographical distribution at the time of human settlement (McFadgen 1989; Smith 1989; Collins *et al.* 2014a,b). Furthermore, recent aDNA analyses of prehistoric mainland NZ specimens show that NZ's mainland was formerly occupied by a widespread endemic lineage (*Phocarctos hookeri* 'NZ'; Collins *et al.* 2014b). Within a few hundred years of human

settlement, however, this distinctive lineage was extinct and subsequently replaced by a previously subantarctic-limited lineage (*P. hookeri* 'subantarctic') (Collins *et al.* 2014a; Fig. 1).

The Chatham Islands are located 650 km east of mainland NZ (Fig. 1). This archipelago was colonized by East Polynesians no earlier than the 13th century AD, although the exact chronology of settlement remains unresolved and reliable evidence for human occupation derives only from 1450 AD onwards (Wilmshusrst *et al.* 2011; Maxwell 2014; Maxwell & Smith 2015). Pinnipeds, including sea lions and fur seals, are abundant in prehuman (<1450 AD) and early prehistoric (ca. 1450–1650 AD) Chatham Islands deposits, but are rare or absent in later sites (>1650 AD; McFadgen 1989; Maxwell 2014; Maxwell & Smith 2015; Fig. 1). Polynesian settlers of the Chatham Islands initially relied heavily on marine megafauna (ca. 50% of the diet), until these food sources apparently declined and/ or went extinct (Maxwell 2014). The local diet then transitioned to one of mixed marine and terrestrial based resources (Maxwell 2014; Maxwell & Smith 2015). This timeline and transition closely parallel a prehistoric
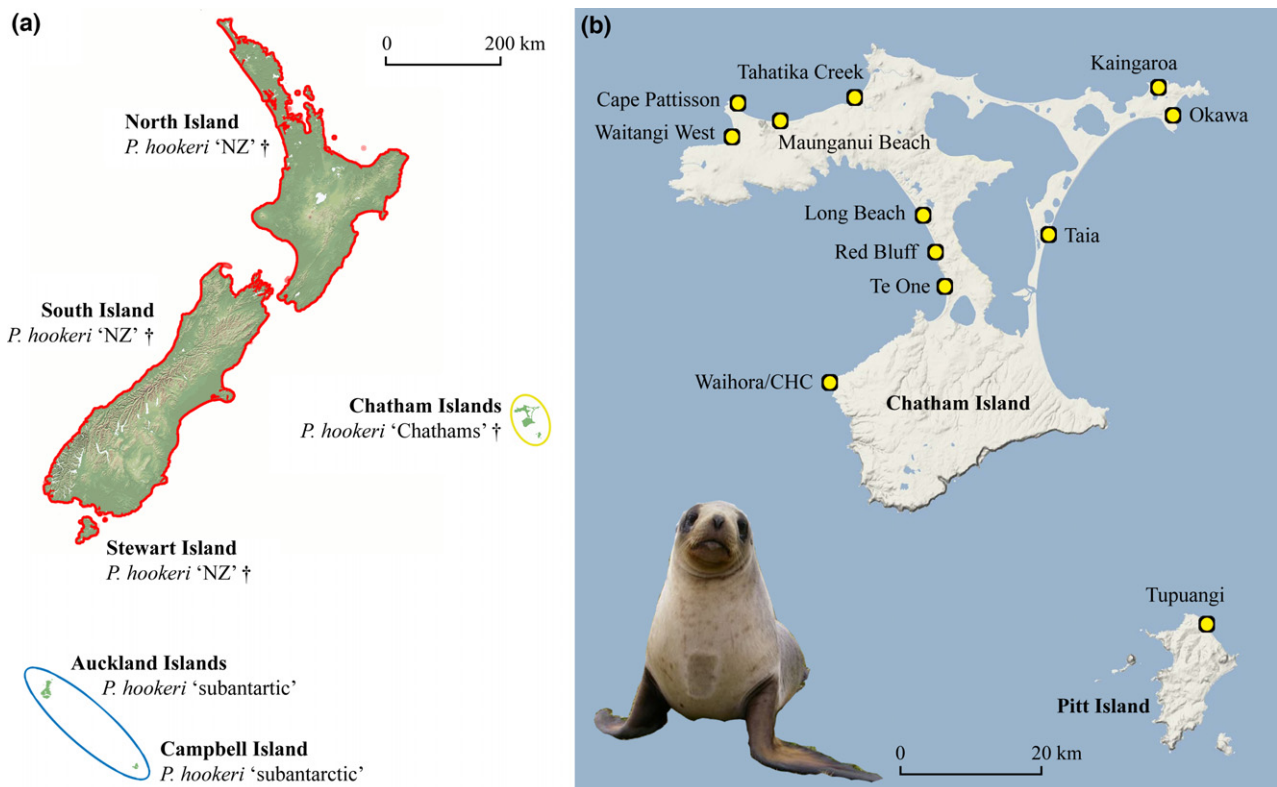
Fig. 1 (A) Distribution of *Phocarctos* sea lions at the time of earliest Polynesian settlement (ca. 1280 AD). † represents extinct lineages. The subantarctic lineage subsequently colonized mainland New Zealand in 1992, after the extinction of the New Zealand lineage. The prehistoric distribution of each lineage is shown in red (NZ), blue (subantarctic) and yellow (Chatham Islands). (B) Location of Chatham Islands *Phocarctos* remains analysed in this study. Each yellow dot represents a prehuman or early prehistoric (1450–1650 AD) site containing sea lions.

faunal transition inferred for mainland NZ (Smith 2011; Rawlence *et al.* 2015b). Pinniped remains are also present at a more recent (historic; *c.*1791–1834 AD) Chatham Island sealing site (Point Durham, CHC; ca. 1800–1830 AD; Fig. 1), although their affinities are unclear (Maxwell 2014; Maxwell & Smith 2015).

Here, we use aDNA analysis and demographic modelling to elucidate the prehistoric demography of Chatham Islands sea lions in comparison with populations on mainland NZ and the subantarctic and test for human-mediated extirpation events in this isolated archipelago. Based on these data, we infer rapid, wholesale extinction of the unique Chatham Islands sea lion within 200 years of human settlement due to low but sustained harvest rates. This study highlights the dramatic impacts even small human population densities can have on marine biodiversity.

Materials and methods

Source and age of specimens

Numerous *Phocarctos* specimens have been identified from prehuman and early prehistoric deposits across the NZ region (Smith 1989, 2011; Anderson 2005; Collins *et al.* 2014a,b). However, sea lion remains are much rarer in later deposits (Smith 2011). Collins *et al.* (2014b) obtained DNA sequences of 35 'NZ' specimens from across the prehuman (<1280 AD) and early prehistoric (1280–1450 AD) range of *Phocarctos* (see Table S1, Supporting information for specimen ages). For *Phocarctos hookeri* 'subantarctic', Collins *et al.* (2014b) obtained sequences from 61 specimens from across the modern (Auckland and Campbell Islands, mainland NZ) and prehistoric (Auckland Islands, 1280–1450 AD) range (see Table S1, Supporting information for specimen ages). In the current study, we expand the *Phocarctos* data set through whole mitogenome sequencing (*n* = 3; samples in Table S1, Supporting information) and by analysing 27 new sea lion D-loop sequences (189 bp) of individuals from the Chatham Islands (out of 70 specimens attempted). Our study focuses on the prehistoric demography of Chatham Islands sea lions and how this compares to mainland NZ and subantarctic populations.

Pinniped remains from prehuman (<1450 AD), early prehistoric (ca. 1450–1650 AD) and historic (1791–1830 AD) sites on the Chatham Islands (*n* = 70) were obtained from museum collections (Fig. 1; Table S1, Supporting information). Equivalent remains are apparently absent from late prehistoric sites on the Chatham Islands (McFadgen 1989; Maxwell 2014; Maxwell & Smith 2015). Specimen ages were inferred by their association with well-dated prehuman and early prehistoric remains (Fig. 1), or from associated material culture and faunal assemblage being consistent with early prehistoric age (e.g. Cape Pattisson, Tupuangi Beach; Fig. 1; McFadgen 1989; Maxwell 2014; Maxwell & Smith 2015). While these latter archaeological sites can be classified as 'early prehistoric', they may also contain prehuman remains up to 6000 years old (e.g. Millener 1999). Historical specimens are from well-dated historic sites (ca. 1791–1834 AD; Maxwell & Smith 2015). To ensure independence of samples, only common elements of the left or right orientation (e.g. left femora only at a given site) were sampled from an individual deposit, or bones were sampled from different stratigraphic units within a site (e.g. layers, squares). Specimens were sampled from sites that have previously yielded aDNA (e.g. Mitchel *et al.* 2014; Rawlence *et al.* 2014; Wood *et al.* 2014). In instances where morphological and genetic identifications of pinniped bones conflicted, morphological re-analysis of specimens was conducted using comparative collections in the Museum of New Zealand Te Papa Tongarewa, Canterbury Museum and Otago Museum.

Ancient DNA extraction, amplification and sequencing

Ancient DNA extractions and PCR set-up were carried out in a purpose-built aDNA laboratory physically isolated from other molecular laboratories (Knapp *et al.* 2012a). Strict aDNA procedures were followed to minimize contamination of samples with exogenous DNA and to authenticate aDNA sequences (Knapp *et al.* 2012a) including the use of negative extraction (i.e. no sample) and PCR (i.e. no template DNA) controls. No Chatham Island sea lion specimens had been analysed in the laboratory prior to this study. DNA was extracted following Rohland *et al.* (2010) and up to 189 bp of mtDNA D-loop was amplified and sequenced in two small overlapping fragments following Collins *et al.* (2014b).

The short D-loop region was independently amplified at least twice from each aDNA extract, and the resulting amplicons sequenced bidirectionally from independent PCR products. When singletons, or an inconsistency between sequences from an individual, were observed, likely due to post-mortem DNA damage (G-A and C-T transitions), additional PCRs and bidirectional sequencing were conducted, and a majority-rule consensus was applied to the independent replicates (Brotherton *et al.* 2007).

Phylogenetic analysis of D-loop sequences

Phocarctos D-loop sequences were recovered from 27 Chatham Islands individuals (43 out of 70 individuals initially sampled did not produce amplifiable DNA or were misidentified fur seals; Table S1, Supporting

information). Of these 27, 26 were prehuman (<1450 AD) to early prehistoric (1450–1650 AD) in age and one historic (1791–1834 AD) (Fig. 1; Table S1, Supporting information). Contiguous sequences (GenBank Accession nos. KX271755-KX271781) were constructed using Sequencher (Gene Codes) from high-quality sequence reads and aligned in MEGA 4.0 (Kumar *et al.* 2004) with published *Phocarctos* sequences (Collins *et al.* 2014b) representing the geographical and temporal ranges of the extinct 'NZ' (<1450 AD) and 'subantarctic' lineages. This data set included 35 'NZ' sequences representing 14 haplotypes (KJ588766-KJ588778, KX388142); and 61 'subantarctic' sequences (comprising ancient Auckland Islands, modern Auckland and Campbell Islands and recently recolonized NZ mainland specimens) representing three haplotypes (KJ648152-KJ648154). The South American sea lion (*Otaria byronia*; HM467643), New Zealand fur seal (*Arctocephalus forsteri*; NC004023) and Australian sea lion (*Neophoca cinerea*; NC_008419) were used as out-groups, resulting in a 126-specimen data set.

As is typical when using aDNA, our data contained some partial (i.e. missing sequence data) D-loop sequences (12/27 specimens). These sequences were conservatively assigned to haplotypes based on comparison with full-length 189-bp D-loop sequences of all three *Phocarctos* lineages (a total of 29 haplotypes were included, including the three out-groups). The partial sequences share unique mutations consistent with them being part of the Chatham Islands clade and not the NZ or subantarctic clades (i.e. no mutations unique to the NZ or subantarctic clades were found in the partial sequences from the Chatham Islands). Following replicated amplifications, only one ambiguous site (R, likely representing post-mortem DNA damage, in NRO1004 Chatham Island) remained. These data imply that DNA damage is unlikely to contribute substantially to mtDNA haplotype diversity estimates for prehistoric specimens. By themselves, these ambiguous sites were not considered to represent new haplotypes but were instead assigned conservatively to existing haplotypes.

Phylogenetic analysis to establish the relationship between the three sea lion lineages and haplotypes herein was performed on the 29 exemplar haplotype data set (Fig. S1, Supporting information) using BEAST v1.8 (Drummond *et al.* 2012). The most appropriate model of nucleotide substitution was HKY+I+G (using the Akaike information criterion of MODELTEST 3.7; Posada & Crandall 1998). Bayesian analysis was performed using a Yule process speciation prior (Yule 1925; Gernhard 2008). Bayes factor (BF) comparison of the likelihood (1000 bootstrap replicates) for Yule vs. birth–death (Gernhard 2008) speciation priors indicated strong but not decisive support for the more complex

model (Yule BF 0.015; birth–death BF 66.80; Newton & Raftery 1994; Kass & Raftery 1995; Suchard *et al.* 2001). However, as the general topology of both trees was the same, the less complex model is presented (Fig. S1, Supporting information). For each prior, three independent runs were conducted, each consisting of 50 million MCMC generations, sampling tree parameters every 1000 generations, with a burn-in of 25%. Analysis of phylogenetic convergence was assessed in TRACER v1.5, and results visualized in FIGTREE v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

To examine temporal population continuity of the three sea lion lineages, *Phocarctos* sequences were visualized as a heterochronous parsimony network using Tempnet (Prost & Anderson 2011; Fig. 2). Partial sequences were conservatively binned to matching full-length (189 bp) haplotypes, as TempNet overestimates haplotype diversity in the presence of missing or ambiguous data. The out-group sequences and the historic vagrant *P. hookeri* 'subantarctic' individual from the Chatham Islands (PhoCHI1, Chatham Island) were excluded from the TempNet, resulting in a 122-specimen data set. Sequences were divided into three time bins (representing extinction times) based on direct or associated radiocarbon dates, or material culture and/or faunal assemblage from the same site/layer consistent with a given prehistoric period (see Source and age of specimens; Table S1, Supporting information): <1450 AD (ancient *P. hookeri* 'subantarctic' from Auckland Islands; *P. hookeri* 'NZ'); <1650 AD (*P. hookeri* 'Chatham Islands'); and modern (*P. hookeri* 'subantarctic' from the Auckland and Campbell Islands, and mainland NZ). Haplotype diversity (*H*) was calculated using R package pegas.

Whole mitogenome sequencing

Whole mitogenome sequencing was used to calculate genetic divergences among Chatham Island, mainland NZ and subantarctic *Phocarctos* lineages and other pinniped taxa. One 'mainland NZ' (GenBank Accession no. KX388143-KX388144) and one 'Chatham Island' individual were sequenced and compared with a published 'subantarctic' mitogenome (GenBank Accession no. NC_008418). Double-stranded sequencing libraries were prepared directly from aDNA extracts following Orton *et al.* (2013). Hybridization capture, following Maricic *et al.* (2010), was used to target the mitogenome for sequencing. Baits were constructed using modern *P. hookeri* 'subantarctic' DNA. See Supporting information for full methodology of sequencing library preparation. Enriched libraries were sequenced on the Ion Torrent PGM platform, using a 316 chip. The resulting reads were mapped to the modern *P. hookeri* 'subantarctic' mitogenome, with BOWTIE 2.1.0 (Langmead &
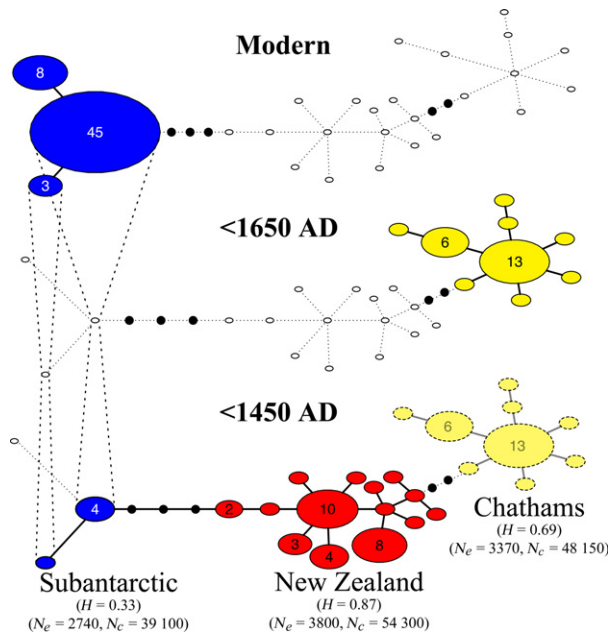
Fig. 2 Temporal network reconstruction of *Phocarctos* sea lion mtDNA D-loop sequences. Haplotypes are represented by circles. Circle size is proportional to haplotype frequency (indicated for common haplotypes). White circles are haplotypes not found within a given extinction period. Black circles represent undetected intermediate haplotypes. Shared haplotypes are connected by vertical dashed lines. Lineage colouring: Blue, subantarctic; red, mainland NZ; yellow, Chatham Islands. H = haplotype diversity; N_e = effective population size; N_c = census population size. The yellow-dashed Chatham Island haplotypes in the <1450 AD extinction period represent the uncertainty in the dating of these specimens.

Salzberg 2012) using the -n alignment mode and allowing for two mismatches, as is typical for aDNA. Additionally, MAPDAMAGE 2.0 (Ginolhac *et al.* 2011) was used to lower the quality score of likely damaged aDNA reads, using the '-rescale' option, and confirm that the reads displayed characteristic aDNA damage patterns. See Supporting information for full details of bioinformatic processing and sequence analysis. *Phocarctos* and published pinniped mitogenomes (Arnason *et al.* 2006) were aligned, and phylogenetic analyses were performed using BEAST 2.1.3, with a Yule process speciation prior (Yule 1925; Gernhard 2008) and the GTR+I+G model of nucleotide substitution (Fig. 3). See Supporting information for full methodology of mitogenome phylogenetic analyses.

BayeSSC and individual-based modelling of anthropogenic mortality

Bayesian Serial SimCoal (BayeSSC; Excoffier *et al.* 2000; Anderson *et al.* 2005) was used to model the effective

population size (N_e) of *P. hookeri* 'Chatham Islands' relative to the extinct 'mainland NZ' and 'subantarctic' lineages at the time of human arrival (1280 AD for the subantarctic and mainland NZ lineage, and 1450 AD for the Chatham Island lineage). Each of the three populations was modelled as having a constant effective size after splitting from an ancestral population of constant size. All the population size priors were broad and uninformative (log-uniform from 100 to 1 000 000), as were the split times (log-uniform from the age of the oldest specimen to 1 000 000 years before present) to prevent biasing of the results. The mutation rate was also allowed to vary from 1 to 25% substitutions/site/million years. Due to uncertainty about the age of the Chatham Island individuals, five variations of this model were analysed where the tips ages were (i) 500 years BP, (ii) 3000 years BP, (iii) 6000 years BP or evenly spread from (iv) 500 to 3000 years BP, or (v) 500 to 6000 years BP. For full details, see supporting information (Table S2; Fig. S2, Supporting information). The short region of D-loop sequenced did not provide sufficient power to perform a Bayesian skyline analysis (Drummond *et al.* 2005) or equivalent demographic modelling in BAYESSC (data not shown), as both Tajima's D and mismatch distributions were statistically indistinguishable from neutral.

The estimates of N_e derived above allowed the calculation of census population size (N_c). The N_e:N_c ratio is a species-specific estimate of 0.14 (based off nuclear microsatellite data; Robertson 2015a), multiplied by two to include both males and females. This estimate of 0.14 is similar to that observed in other mammalian species (Frankham 1995) and likely conservative for otariids. The polygynous mating system of sea lions causes relatively few males to contribute genetically in each generation, driving the N_e:N_c ratio lower for nuclear genes, but not the mtDNA D-loop used in this study. Estimates of N_c should be treated as upper estimates, with resulting anthropogenic mortality rates lower than those reported sufficient to cause lineage extinction.

These census estimates were then used in a demographic individual-based model (IBM) to determine the amount of anthropogenic mortality likely to lead to extirpation within 200 years. Sea lion populations are modelled as a two-sex, 23-age pyramid with a corresponding Leslie matrix, given a 250-year burn-in period to reach stability, and are then subjected to 1000 randomly selected levels of mortality/human/year. Demographic stochasticity is included by having sex assigned randomly at birth, and harvest stochasticity is included by allowing the actual number of kills each year to vary around the randomly selected average. This database of 1000 population trajectories is then used to determine the level of anthropogenic mortality needed to cause
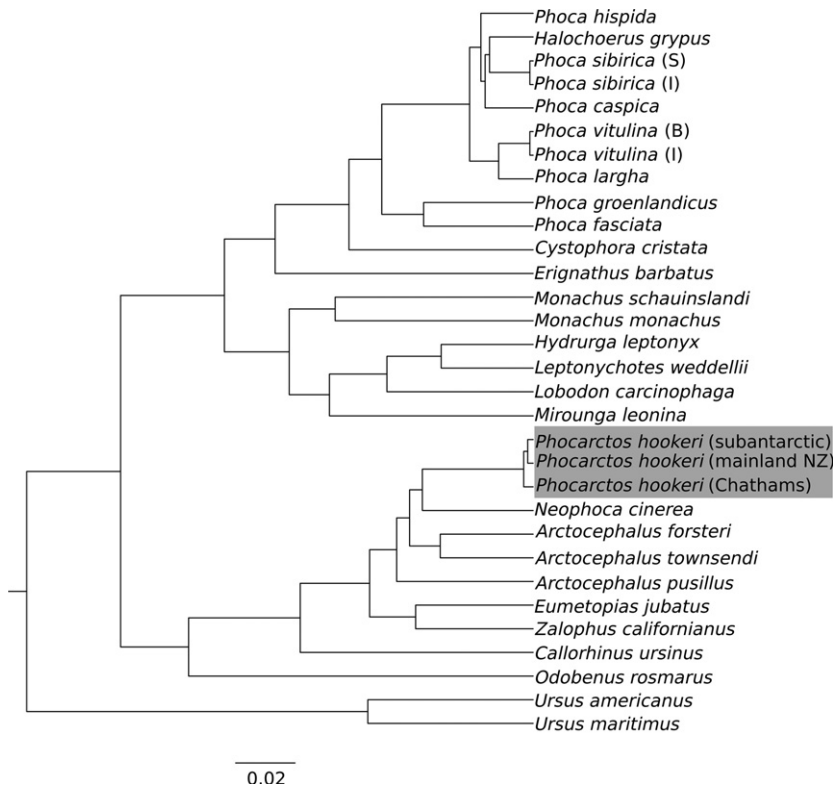
Fig. 3 Bayesian maximum clade credibility tree of published pinniped mtDNA sequences and ancient 'mainland' and 'Chatham Islands' *Phocarctos hookeri* (this study), constructed from 12 mitochondrial genome coding regions (Table S6, Supporting information), constructed using BEAST. The published extant 'subantarctic' *P. hookeri* and the ancient 'mainland' and 'Chatham Islands' *P. hookeri* mitochondrial lineages are highlighted in the grey box. For *Phoca vitulina*, B: Baltic Sea; I: Iceland. For *Phoca sibirica*, S: Sweden, I: Iceland. All posterior probabilities were >0.99.

the extinction of each *Phocarctos* lineage (i.e. the number of individuals harvested/year to give a 5, 50 and 95% probability of extinction within 200 years). A similar process was used to establish the risk of extinction to the modern subantarctic population based on the current population size estimate of 9880 individuals, based on pup counts (Geschke & Chilvers 2009). This custom built IBM model is fully described in Supporting information.

Results

Ancient DNA and phylogenetic analysis

Phocarctos D-loop sequences were recovered from 27 of the 70 Chatham Islands pinniped specimens, sourced from 10 prehuman (<1450 AD) and early prehistoric sampling localities (1450–1650 AD; Figs 1 and 2). The other 43 specimens were either attributable to *Arctocephalus* or did not produce amplifiable DNA (Table S1, Supporting information). All but one of the 27 *Phocarctos* sequences clustered together in a unique Chatham Islands haplogroup (0.94 Posterior Probability; PP) containing nine haplotypes, sister to *Phocarctos hookeri* 'subantarctic' (1.00 PP; three haplotypes) and extinct mainland '*P. hookeri* NZ' (0.77 PP; 14 haplotypes) sister lineages (Figs 2 and S1, Supporting information). The remaining Chathams *Phocarctos* sequence, from a

historic (1791–1834 AD) whole skeleton (PhoCHI1) assumed to be a recent 'subantarctic' vagrant (as no resident *Phocarctos* have been historically recorded on the Chatham Islands; Maxwell 2014; Maxwell & Smith 2015), clustered with *P. hookeri* 'subantarctic' (haplotype SA2; Fig. S1, Supporting information).

Phylogenetic reconstructions based on *Phocarctos* whole mitogenome data similarly supported a sister relationship between mainland NZ and subantarctic lineages (PP = 0.99, Fig. 3), relative to the more divergent Chatham Island lineage. Mitogenomic divergences within *Phocarctos* (0.4–0.7%) are within the order of magnitude of previously reported intraspecific values of other pinniped taxa (within *Phoca sibirica* and *Phoca vitulina*; 0.16–0.34%) but substantially smaller than interspecific values for pinniped sister taxa (*P. sibirica*, *Phoca caspica*; *P. vitulina*, *Phoca largha*; 2.2–2.7%).

Demographic and mortality modelling

The recovery of *Phocarctos* mtDNA from several Chatham Island archaeological specimens (Fig. 1) confirms that this endemic Chatham Island lineage was harvested by humans (Sutton 1980; McFadgen 1989; Maxwell 2014; Maxwell & Smith 2015) during the early prehistoric period (1450–1650 AD). This archaeological material includes a broad ontogenetic range of specimens, suggesting sea lion hunting was not targeted to

any particular size class (or presumably sex). While sea lions were previously reported from the younger (historic) Point Durham (CHC) site (Smith 1977), our genetic and morphological re-analyses indicated that these remains were from *Arctocephalus* rather than *Phocarctos* (data not shown).

BayeSSC modelling suggests a mean female effective population size N_e (averaged across the thirty MLE estimates of the five models, two mutation structures and three summary statistics) of 3370 individuals (census population size N_c = 48 150) for the Chatham Islands, compared with 3800 (54 300) for mainland NZ and 2740 (39 100) for the subantarctic (Fig. 2; Tables S2–S3, Supporting information) compared to the estimated contemporary subantarctic population size of ca. 9880, based on pup counts (Geschke & Chilvers 2009). These estimates suggest the historical subantarctic presealing population size was about four times larger than the current estimate of 9880 individuals.

IBM modelling of the level of harvest required for a 95% probability of extinction within 200 years was 1.05 sea lions/human/year for the prehistoric mainland NZ lineage (Figs 4 and S5; Table S3, Supporting information). Making the conservative assumption that the Chatham Island human population grew to 2000 individuals within 200 years of colonization (Maxwell 2014), 0.97 sea lions/human/year would be sufficient to eliminate the lineage with 95% probability, while a human population of 6000 (Maxwell 2014) would require harvest of only 0.325 sea lions/human/year (Figs 4 and S5; Table S3, Supporting information). Anthropogenic mortality estimates for the prehistoric subantarctic lineage (at modelled equilibrium *contra.* declining modern population; Chilvers & MacKenzie 2010) ranged from 0.816 sea lions/human/year to 0.736 sea lions/human/year and 0.267 sea lions/human/year, when the human population was modelled using the conservative Chatham Island, NZ, and realistic Chatham Island trajectories, respectively (Figs 4 and S5; Table S3, Supporting information). Put simply, a hunting rate greater than one sea lion/human/year made extinction likely in all sea lion populations under all human population growth scenarios. The same modelling approach on the declining modern subantarctic population of 9880 sea lions, based off pup counts (Geschke & Chilvers 2009), suggests a 95% probability of extinction in <50 years if 410 sea lions or more are killed annually (Fig. 5; Table S3, Supporting information).

Discussion

Phylogeographic analyses and Bayesian modelling of prehistoric specimens indicate that, at the time of human settlement, the Chatham Islands supported a large, genetically distinct population of *Phocarctos* sea lions. The sudden extinction of this endemic lineage is apparently attributable to hunting, along with other anthropogenic impacts (Sutton 1980; McFadgen 1989; Maxwell & Smith 2015). Given that the divergent Chatham Islands lineage likely persisted *in situ* through recent glacial–interglacial transitions, relatively minor climatic changes during recent centuries are unlikely to have contributed substantially to its extirpation (Allen 2012). This apparently rapid human-mediated extirpation event presents a close parallel to the previously reported mainland NZ *Phocarctos* extirpation (Collins *et al.* 2014b). Together these findings highlight that extant sea lion populations retain only a fraction of the genus' substantial prehistoric mitochondrial genetic diversity. Whole mitogenomic analyses confirm the divergent phylogenetic placement of the Chatham Island lineage relative to mainland NZ and subantarctic *Phocarctos*. The genetic distance between the *Phocarctos* mitochondrial lineages may be greater than the genetic distance between *Phocarctos* populations when nuclear regions of the genome are assessed as *Phocarctos* sea lions display female philopatry (Chilvers & Wilkinson 2008), while males, in contrast, are frequently observed to move between breeding areas (Robertson *et al.* 2006). Future studies should further assess the genomewide diversity (and taxonomic status) of these extinct *Phocarctos* lineages.

Recent research suggests sea lions were likely extinct on the Chatham Islands by 1650 AD, within a few centuries of human contact (Figs 1 and 2; McFadgen 1989; Maxwell 2014; Maxwell & Smith 2015; this study). Indeed, archaeological data from early prehistoric sites suggest that sea lions and fur seals together were a dominant dietary energy source for Polynesians, with estimates as high as ca. 15–40% and 50% for *Phocarctos* in NZ and the Chatham Islands, respectively (Smith 2011; Maxwell 2014). These percentages are broadly congruent with our simulations. Contemporary hunter-gatherer societies acquire ca. 19–40% of their energy from protein (Cordian *et al.* 2000), which is equivalent to approximately 45 kg of protein/year for an adult averaging an intake of 2000 calories/day. An adult sea lion contains approximately 30 kg of protein (Trites & Jonker 2000; Correa *et al.* 2014), implying that if Polynesians obtained ca. 50% of their protein intake from sea lions, the harvest rate would need to be ca. 0.67 sea lions/human/year. This value is within the range of simulated harvest rates likely to lead to sea lion extinction in all modelled scenarios (Table S3, Supporting information; Fig. 4). This finding provides additional evidence for rapid anthropogenic overkill of large vertebrate populations by small resident human populations (e.g. Holdaway *et al.* 2014; Perry *et al.* 2014).
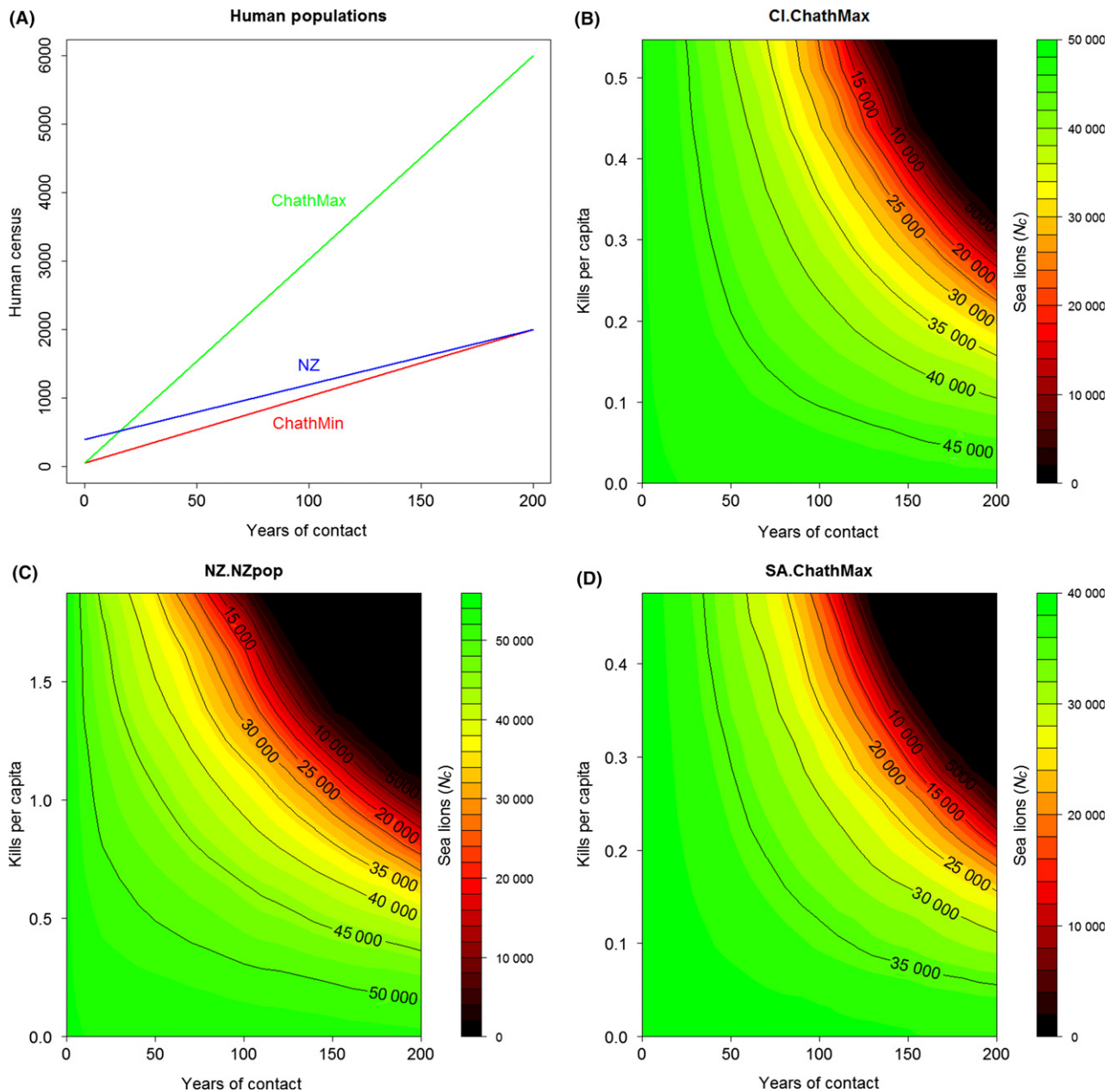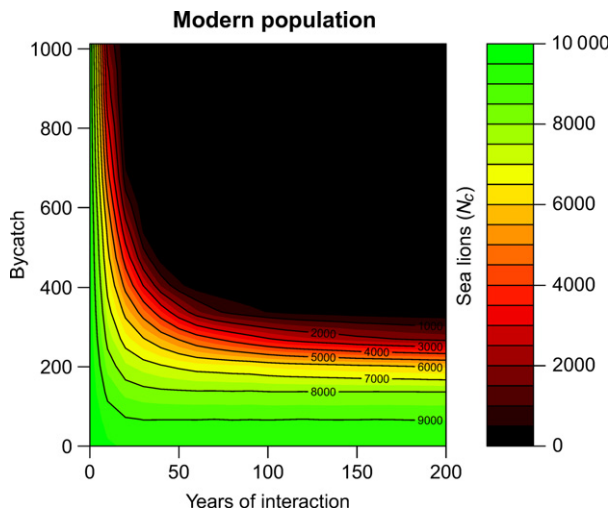
Fig. 4 The combined population trajectories of 1000 population simulations. Given various scenarios of human population growth (A), each *Phocarctos* population was simulated for 200 years postcontact at an average number of sea lions harvested/human/year. Even in the absence of other causes of mortality, extinction within 200 years was likely at kill rates higher than one sea lion/human/year in all three populations under all human growth scenarios: Chatham Islands (B), New Zealand (C) and subantarctic (D). For additional scenarios, see Fig. S5 (Supporting information).

The Chatham Islands sea lion population was likely concentrated along soft shorelines similar to the sandy beaches where breeding colonies are found today. Such habitats are also favoured by humans, which may help to explain their rapid demise (Smith 1977; Sutton 1980). Our demographic modelling indicates that even low levels of persistent harvesting would have driven the inevitable extinction of this large, low-fecundity species,

paralleling numerous megafaunal extinctions (Boessen-kool *et al.* 2009; Stuart 2014; Perry *et al.* 2014; Rawlence *et al.* 2015b). A more refined estimate of sea lion extinction time is not presently possible due to the broad *Phocarctos* foraging range (Robertson *et al.* 2006) encompassing different oceanic ^{14}C pools of varying ages. This results in wide marine reservoir corrections (69 ± 14 to 244 ± 40 years BP) for radiocarbon dates on

Fig. 5 The combined population trajectories of 1000 population simulations for the level of annual mortality required to cause the extinction of the declining modern subantarctic *Phocarctos* population (9880 individuals based on pup counts; Geschke & Chilvers 2009) within a 200-year period.

marine mammal bones from the Chatham Islands (Petchey *et al.* 2008).

The modelled mortality estimates should be treated as a minimum estimate, as human population size would likely have increased exponentially following colonization (from an estimated founding population size of ca. 400 and ca. 50 individuals for NZ and the Chatham Islands, respectively; Whyte *et al.* 2005; Knapp *et al.* 2012b; Maxwell 2014), requiring a substantially greater harvesting level of *Phocarctos* populations due to the high reliance on sea lions for protein (Smith 2011; Maxwell 2014). Additionally, in many cases, a single female kill would have resulted in an additional 1–2 deaths if pregnancies and dependant pups are taken into account.

Overall, this study highlights the rapid extinction of previously diverse and abundant marine megafaunal lineages, indicating dramatic anthropogenic biodiversity losses in coastal ecosystems. Demographic and mortality modelling suggests that sea lion populations can withstand only low levels of hunting and/or mortality (ca. 1–2% of total population; see review by Brook *et al.* 2013), with extinction all but guaranteed if this level is exceeded. Modelling approaches such as used in this study have the potential to predict tipping points in advance (Scheffer *et al.* 2009; Scheffer 2010). These findings also have implications for conservation management of the remaining sea lion populations (*Phocarctos hookeri* 'subantarctic' on mainland NZ, Auckland and Campbell Islands). *Phocarctos* sea lions are listed as 'endangered' by the IUCN Red List (Chilvers 2015) and

are in decline on the subantarctic Auckland Islands (50% decline in pup production since 1998), with fisheries bycatch and resource competition due to commercial fisheries as likely drivers (Robertson & Chilvers 2011). Current estimates of anthropogenic mortality based on bycatch and fisheries interaction data, and allowed bycatch limits (1993–2013: average 81 sea lion deaths/year; range: 63–150/year; Robertson & Chilvers 2011; AEBAR 2014; Robertson 2015a,b), are up to 15–37% of our modelled annual mortality (410 sea lions/year) required to cause lineage extinction in <50 years (not accounting for other causes of mortality, e.g. predation, disease, old age; Fig. 5). The actual percentage of annual mortality made up by bycatch may be as high at 30–74%, given recent reports that up to half the instances of bycatch go unreported (Meyer and Robertson, unpublished data). As suggested by other studies (e.g. Robertson & Chilvers 2011; Meyer *et al.* 2015; but see Roberts & Doonan 2014), our tests for human-mediated extirpation have highlighted that current levels of anthropogenic mortality on remaining *Phocarctos* populations have important implications for species persistence.

Acknowledgements

References

AEBAR (2014) Aquatic environment and biodiversity annual review 2014: a summary of environmental interactions between fisheries and the aquatic environment. Fisheries Management Science Team, Wellington, New Zealand. Available from http://www.maf.govt.nz.

Allen MS (2012) Molluscan foraging efficiency and patterns of mobility amongst foraging agriculuralists: a case study from northern New Zealand. *Journal of Archaeological Science*, **39**, 295–307.

Anderson A (2005) Subpolar settlement in South Polynesia. *Antiquity*, **29**, 791–800.

Anderson CNK, Ramakrishnan U, Chan YL, Hadly EA (2005) Serial SimCoal: a population genetic model for data from multiple populations and points in time. *Bioinformatics*, **21**, 1733–1734.

Arnason U, Gullberg A, Janke A *et al.* (2006) Pinniped phylogeny and a new hypothesis for their origin and dispersal. *Molecular Phylogenetics and Evolution*, **41**, 345–354.

Boessenkool S, Austin JJ, Worthy TH *et al.* (2009) Relict or colonizer? Extinction and range expansion of penguins in

southern New Zealand. *Proceedings of the Royal Society London Series B: Biological Sciences*, 276, 815–821.

Brook BW, Ellis EC, Perring MP, Mackay AW, Blomqvist L (2013) Does the terrestrial biosphere have planetary tipping points? *Trends in Ecology and Evolution*, 28, 396–401.

Brotherton P, Endicott P, Sanchez JJ *et al.* (2007) Novel high-resolution characterization of ancient DNA reveals C-U type base modificaiton events as the sole cause of post mortem miscoding lesions. *Nucleic Acids Research*, 35, 5717–5728.

Chilvers BL (2015) *Phocarctos hookeri*. The IUCN red list of threatened species 2015: e. T17026A1306343. Available from http://dx.doi.org/10.2305/IUCN.UK.2015-2.RLTS.T17026A1306343.en.

Chilvers BL, MacKenzie DI (2010) Age- and sex-specific survival estimates incorporating tag loss for New Zealand sea lions, *Phocarctos hookeri*. *Journal of Mammalogy*, 91, 758–767.

Chilvers BL, Wilkinson IS (2008) Philopatry and site fidelity of New Zealand sea lions (*Phocarctos hookeri*). *Wildlife Research*, 35, 463–470.

Collins CJ, Rawlence NJ, Worthy TH *et al.* (2014a) Pre-human New Zealand sea lion (*Phocarctos hookeri*) rookeries in coastal New Zealand. *Journal of the Royal Society of New Zealand*, 44, 1–16.

Collins CJ, Rawlence NJ, Prost S *et al.* (2014b) Extinction and recolonisation of coastal megafauna following human arrival in New Zealand. *Proceedings of the Royal Society London Series B: Biological Sciences*, 281, 20140097.

Cordian L, Miller JB, Eaton SB, Mann N, Holt SHA, Speth JD (2000) Plant-animal subsistence ratios and macronutrient energy estimations in worldwide hunter-gather diets. *American Journal of Clinical Nutrition*, 71, 682–692.

Correa L, Rea LD, Bentzen R, O'Hara TM (2014) Assessment of mercury and selenium tissular concentrations and total mercury body burden in 6 Steller sea lion pups from the Aleutian Islands. *Marine Pollution Bulletin*, 82, 175–182.

De Bruyn M, Hall BL, Chauke LF, Baroni C, Koch PL, Hoelzel AR (2009) Rapid response of a marine mammal species to Holocene climate and habitat change. *PLoS Genetics*, 5, e1000554.

Drummond AJ, Rambaut A, Shapiro B, Pybus OG (2005) Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular Biology and Evolution*, 22, 1185–1192.

Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, 29, 1969–1973.

Excoffier L, Novembre J, Schneider S (2000) SIMCOAL: a general coalescent program for simulation of molecular data in interconnected populations with arbitrary demography. *Journal of Heredity*, 91, 506–509.

Frankham R (1995) Effective population size/adult population size ratios in wildlife: a review. *Genetics Research*, 66, 95–107.

Gernhard T (2008) The conditioned reconstructed process. *Journal of Theoretical Biology*, 253, 769–778.

Geschke K, Chilvers BL (2009) Managing big boys: a case study on remote anaesthesia and satellite tracking of adult male New Zealand sea lions (*Phocarctos hookeri*). *Wildlife Research*, 36, 666–674.

Ginolhac A, Rasmussen M, Gilbert MTP, Willerslev E, Orlando L (2011) mapDamage: testing for damage patterns in ancient DNA sequences. *Bioinformatics*, 27, 2153–2155.

Holdaway RN, Allentoft ME, Jacomb C, Oskam CL, Beavan NR, Bunce M (2014) An extremely low-density human population exterminated New Zealand moa. *Nature Communications*, 5, 5436.

Kass RE, Raftery AE (1995) Bayes Factors. *Journal of the American Statistical Association*, 90, 773–795.

Knapp M, Clarke AC, Horsburgh KA, Matisoo-Smith EA (2012a) Setting the stage - building and working in an ancient DNA laboratory. *Annals of Anatomy*, 194, 3–6.

Knapp M, Horsburgh KA, Prost S *et al.* (2012b) Complete mitochondrial DNA genome sequences from the first New Zealanders. *Proceedings of the National Academy of Sciences USA*, 109, 18350–18353.

Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics*, 5, 150–163.

Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. *Nature Methods*, 9, 357–359.

Maricic T, Whitten M, Pääbo S (2010) Multiplexed DNA sequence capture of mitochondrial genomes using PCR products. *PLoS ONE*, 5, e14004.

Maxwell JJ (2014) The Moriori: the integration of arboriculture and agroforestry in an east Polynesian society. Unpublished PhD Thesis, University of Otago.

Maxwell JJ, Smith IWG (2015) A re-assessment of settlement patterns and subsistence at Point Durham, Chatham Island. *Archaeology in Oceania*, 50, 162–174.

McFadgen BG (1989) Archaeology and Holocene sand dune stratigraphy on Chatham Island. *Journal of the Royal Society of New Zealand*, 24, 17–44.

Meyer S, Robertson BC, Chilvers BL, Krkošek M (2015) Population dynamics reveal conservation priorities of the threatened New Zealand sea lion *Phocarctos hookeri*. *Marine Biology*, 162, 1587–1596.

Millener PR (1999) The history of the Chatham Islands' bird fauna of the last 7000 years – a chronicle of change and extinction. *Smithsonian Contributions to Palaeobiology*, 89, 85–109.

Mitchel KJ, Wood JR, Scofield RP, Llamas B, Cooper A (2014) Ancient mitochondrial genome reveals unsuspected taxonomic affinity of the extinct Chatham duck (*Pachyanas chathamica*) and resolves divergence times for New Zealand and sub-Antarctic brown teals. *Molecular Phylogenetics and Evolution*, 70, 420–428.

Newton MA, Raftery AE (1994) Approximate Bayesian inference with the weighted likelihood bootstrap. *Journal of the Royal Statistical Society Series B (Methodological)*, 56, 3–48.

Orton J, Mitchell P, Klein R, Steele T, Horsburgh KA (2013) An early date for cattle from Namaqualand, South Africa: implications for the origins of herding in southern Africa. *Antiquity*, 87, 108–120.

Perry GLW, Wheeler AB, Wood JR, Wilmshurst JM (2014) A high-precision chronology for the rapid extinction of New Zealand moa (Aves, Dirnornithiformes). *Quaternary Science Reviews*, 105, 126–135.

Petchey F, Anderson A, Hogg A, Zondervan A (2008) The marine reservoir effect in the Southern Ocean: an evaluation of extant and new ΔR values and their application to archaeoogical chronologies. *Journal of the Royal Society of New Zealand*, 38, 243–262.

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. *Bioinformatics*, 14, 817–818.

Prost S, Anderson CNK (2011) TempNet: a method to display statistical parsimony networks for heterochronous DNA sequence data. *Methods in Ecology and Evolution*, **2**, 663–667.

Rawlence NJ, Till CE, Scofield RP *et al.* (2014) Strong phylogeographic structure in a sedentary seabird, the Stewart Island Shag (*Leucocarbo chalconotus*). *PLoS ONE*, **9**(e90769), 1–9.

Rawlence NJ, Kennedy M, Anderson CNK *et al.* (2015a) Geographically contrasting biodiversity reductions in a widespread New Zealand seabird. *Molecular Ecology*, **24**, 4605–4616.

Rawlence NJ, Perry GLW, Smith IWG *et al.* (2015b) Radiocarbon-dating and ancient DNA reveal rapid replacement of extinct prehistoric penguins. *Quaternary Science Reviews*, **112**, 59–65.

Roberts J, Doonan I (2014) NZ sea lion: demographic assessment of the causes of decline at the Auckland Islands: demographic model options - correlative assessment. Technical report, NIWA - National Institute for Aquatic and Atmosphere Research, Wellington, New Zealand.

Robertson BC (2015a) Is management limiting the recovery of the New Zealand sea lion *Phocarctos hookeri*? *Polar Biology*, **38**, 539–546.

Robertson BC (2015b) Comment on "Review of research and assessments on the efficacy of sea lion exclusion devices in reducing the incidental mortality of New Zealand sea lions *Phocarctos hookeri* in the Auckland Islands squid trawl fishery". *Fisheries Research*, **165**, 127–129.

Robertson BC, Chilvers BL (2011) The population decline of the New Zealand sea lion *Phocarctos hookeri*: a review of possible causes. *Mammal Review*, **41**, 253–275.

Robertson BC, Chilvers BC, Duignan PJ, Wilkinson IS, Gemmell NJ (2006) Dispersal of breeding, adult male *Phocarctos hookeri*: implications for disease transmission, population management and species recovery. *Biological Conservation*, **127**, 227–236.

Rohland N, Siedel H, Hofreiter M (2010) A rapid column-based ancient DNA extraction method for increased sample throughput. *Molecular Ecology Resources*, **10**, 677–683.

Scheffer M (2010) Complex systems: forseeing tipping points. *Nature*, **467**, 411–412.

Scheffer M, Bascompte J, Brock WA *et al.* (2009) Early-warning signals for critical transitions. *Nature*, **461**, 53–59.

Smith IWG (1977) Prehistoric fur seal exploitation on the south west coast of Chatham Island. Working Papers in Chatham Island Archaeology No. 7.

Smith IWG (1989) Maori impact on the marine megafauna: pre-European distributions of New Zealand sea mammals. In: *In saying so doesn't make it so: papers in honour of B. Foss Leach* (ed. Sutton DG), pp. 76–108. Archaeological Association Monograph 17, Dunedin, New Zealand.

Smith IWG (2011) Estimating the magnitude of pre-European Maori marine harvest in two New Zealand study areas. New Zealand Aquatic Environment and Biodiversity Report No. 82.

Stuart AJ (2014) Late Quaternary megafaunal extinctions on the continents: a short review. *Geological Journal*, **50**, 338–363.

Suchard MA, Weiss RE, Sinsheimer JS (2001) Bayesian selection of continuous-time Markov Chain Evolutionary Models. *Molecular Biology and Evolution*, **18**, 1001–1013.

Sutton DG (1980) A culture history of the Chatham Islands. *Journal of the Polynesian Society*, **89**, 67–94.

Trites AW, Jonker RAH (2000) Morphometric measurements and body condition of healthy and starving Steller sea lion pups (*Eumetopias jubatus*). *Aquatic Mammals*, **26**, 151–157.

Wanner H, Beer J, Butikofer J *et al.* (2008) Mid- to Late Holocene climate change: an overview. *Quaternary Science Reviews*, **27**, 1791–1828.

Whyte AL, Marshall SJ, Chambers GK (2005) Human evolution in Polynesia. *Human Biology*, **77**, 157–177.

Wilmshurst JM, Anderson AJ, Higham TFG, Worthy TH (2008) Dating the late prehistoric dispersal of Polynesians to New Zealand using the commensal Pacific rat. *Proceedings of the National Academy of Sciences USA*, **105**, 7676–7680.

Wilmshusrst JM, Hunt TL, Lipo CP, Anderson AJ (2011) High-precision radiocarbon dating shows recent and rapid initial human colonisation of East Polynesia. *Proceedings of the National Academy of Sciences USA*, **108**, 1815–1820.

Wood JR, Mitchell KJ, Scofield RP *et al.* (2014) An extinct nestorid parrot (Aves, Psittaciformes, Nestoridae) from the Chatham Islands, New Zealand. *Zoological Journal of the Linnean Society*, **172**, 185–199.

Worthy TH, Holdaway RN (2002) *Lost World of the Moa*. Canterbury University Press, Christchurch.

Yule GU (1925) A mathematical theory of evolution, based on the conclusions of Dr. J.C. Willis. *Philosophical Transactions of the Royal Society of London Biological Series*, **213**, 21–87.

Data accessibility

DNA sequences: Genbank Accession nos. KX271755-KX271781, KX388143-KX388144. Sequence alignments, code implementing the individual-based mortality model, and tree and BAYESSC input files have been accessioned onto Dryad (http://dx.doi.org/10.5061/dryad.118vg).

Supporting information

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

Appendix S1 Materials and Methods: whole mitogenome sequencing, BayeSSC modelling, and individual based modelling of anthropogenic mortality estimation.

Table S1 Modern and prehistoric *Phocarctos* sea lion specimens used for analysis.

Table S2 Results of the BayeSSC model simulations.

Table S3 Average number of sea lions harvested/human/year to cause the extinction of *Phocarctos* within 200 years given possible human demographic scenarios.

Table S4 Summary statistics describing of results from Ion Torrent sequencing reads for whole mitogenomes of *Phocarctos* sea lions.

Table S5 Number of sequence reads supporting and conflicting with the *Phocarctos* mitogenome consensus sequence.

Table S6 Genbank accession numbers for pinniped complete mitochondrial genome and coding region sequences used in the phylogenetic analysis of *Phocarctos* sea lion mitogenomes.

Fig. S1 The Bayesian maximum clade credibility tree of *Phocarctos* sea lions from an analysis of a single individual of each haplotype.

Fig. S2 Demographic model of *Phocarctos* sea lion populations analysed in BayeSSC.

Fig. S3 Posterior density distribution of the substitution rate-estimate derived from BayeSSC simulations, when the rate is given a wide, uninformative uniform prior.

Fig. S4 An example of the initial population pyramid and survivorship curves for *Phocarctos hookeri*.

Fig. S5 The combined population trajectories of 1000 simulations, given various scenarios of human population growth and hunting over a 200 year period.

Fig. S6 Results of 1000 simulations of the Chatham Island sea lion population with the ChathMax human population trajectory using logistic regression.

Fig. S7 Fragment length distribution of mapped reads to the *Phocarctos hookeri* mitochondrial genome from sequencing libraries.

Fig. S8 Coverage plots of Ion Torrent reads mapped to complete *Phocarctos hookeri* mitochondrial genome.