The critically endangered Maui’s dolphin (Cephalorhynchus hectori maui) and the endangered Hector’s dolphin (C. h. hectori) are endemic to the coastal waters of New Zealand, where their primary threat is fisheries-related mortality. The Maui’s dolphin is among the most critically endangered cetaceans in the world, with its remnant population primarily concentrated in approximately 140 km along the central west coast of New Zealand’s North Island. Its closely related sister subspecies, the Hector’s dolphin, is more abundant and offers a useful comparison for studying the Maui’s dolphin. My work used genetic tools to examine demographic and genetic parameters relevant for conservation considerations regarding Maui’s and Hector’s dolphins, as well as to build upon past genetic baselines for the purpose of long-term genetic monitoring of these subspecies. Three genetic datasets formed the basis for most analyses: (1) Maui’s 01-07, including 54 Maui’s dolphin individuals sampled between 2001 and 2007 \( (n = 70 \text{ biopsies, 12 beachcast}) \); (2) Maui’s 10-11, including 40 Maui’s dolphin individuals sampled in 2010 and 2011 \( (n = 69 \text{ biopsies, 1 beachcast}) \); and (3) Hector’s CB11-12, including 148 Hector’s dolphin individuals sampled in Cloudy Bay in 2011 and 2012 \( (n = 263 \text{ biopsies}) \). Microsatellite genotypes were used to identify individuals for a genotype recapture abundance estimate of individuals age \( 1^+ (N_{1+}) \) and for the estimation of effective population size \( (N_e) \). Both populations exhibited a high \( N_e \) relative to \( N_{1+} \), consistent with expectations given their life history characteristics and the limited data available for other dolphin species. The abundance of Maui’s dolphins was confirmed to be very low, Maui’s 10-11 \( N_{1+} = 55 \) (95% CL = 48 - 69), and as expected, it had much
lower linkage disequilibrium $N_e$ (61, 95% CL = 29 - 338) than Hector’s CB11-12 ($N_e = 207$, 95% CL = 127 – 447; $N_{I+} = 272$, 95% CL = 236 - 323). The slightly higher $N_e/N_{I+}$ ratio of the Maui’s dolphin compared to the Hector’s dolphin is consistent with a recent decline in the Maui’s dolphin. Although the point estimates of both $N_e$ and $N_{I+}$ decreased between the two Maui’s dolphin datasets (Maui’s 01-07: $N_e = 74$, 95% CL = 37 - 318; $N_{I+} = 69$, 95% CL = 38 - 125), the confidence intervals widely overlapped. Maui’s 10-11 had significantly fewer alleles (average 4 alleles/locus) and lower heterozygosity ($H_o = 0.316$, $H_e = 0.319$) than Hector’s CB11-12 (average 7 alleles/locus, $H_o = 0.500$, $H_e = 0.495$; all $P <0.001$). Interestingly, one microsatellite locus (PPHO104) had anomalously high diversity (31 to 63 alleles) in both Hector’s and Maui’s dolphins and appears to be influenced by diversifying selection. The observed and expected heterozygosity, internal relatedness, and $F_{IS}$ of Maui’s dolphins all showed patterns consistent with a decline of the subspecies, although none differed significantly over the short time interval between the two datasets collected in 2001-07 and 2010-11. The lack of significant decline in any of the parameters analyzed for Maui’s dolphins is not surprising given the low power to detect a low to moderate decline over the short interval (<1 generation) between the two sampling periods.

Compared to minimum viable effective population sizes proposed to guide management decisions, the Maui’s dolphin has declined below the recommended threshold of $N_e = 50$, recently increased to $N_e \geq 100$, thought to be necessary to avoid inbreeding depression in the short term (5 generations, ~65.2 years for Maui’s and Hector’s dolphins). Additionally, both the Maui’s dolphin and Cloudy Bay Hector’s dolphin populations are below the recommended threshold of $N_e = 500$, recently increased to $N_e \geq 1000$, thought to be necessary to preserve long-term evolutionary potential. This is less of a concern for the Cloudy Bay Hector’s population, which is thought to maintain gene flow with neighboring populations. However, for the small, isolated Maui’s dolphin population, inbreeding depression is likely to be an increasing concern. Furthermore, each Maui’s dolphin individual holds a disproportionate amount of the total genetic variation of the subspecies and would represent a disproportionately large demographic and genetic loss if it died before realizing its reproductive potential in the population. There is, however, potential
for genetic restoration by interbreeding with Hector’s dolphins, as genetic monitoring of Maui’s dolphins revealed the first contemporary dispersal of four (two living females, one dead female, one dead male) Hector’s dolphins into the Maui’s dolphin distribution. Two Hector’s dolphins (one dead female neonate, one living male) were also sampled along the North Island’s southwest coast, outside the presumed range of either subspecies. Together, these records provide evidence of long-distance dispersal by Hector’s dolphins (≥400 km) and the possibility of an unsampled Hector’s dolphin population along the southwest coast of the North Island or northern South Island. These results highlight the value of genetic monitoring for subspecies lacking distinctive physical appearances, as such discoveries are not detected by other means but have important conservation implications.

Although the Maui’s dolphin is critically endangered, it is not necessarily doomed to extinction. The subspecies appears to be maintaining an equal sex ratio and connectivity within its remnant range, and the highly diverse locus PPHO104 could potentially offer clues to an inbreeding avoidance mechanism. If Maui’s dolphins interbreed with the recently identified Hector’s dolphin immigrants, it could provide genetic restoration, enhancing chances of long-term survival of the Maui’s dolphin. Continued genetic monitoring and examination of recovered carcasses for phenotypic signs of inbreeding are important for gauging genetic threats to the survival of Maui’s dolphins, as well as determining if any Hector’s dolphin populations appear to be declining toward the critically endangered state of the Maui’s dolphin.

The results of this work contributed to the decision by the New Zealand Department of Conservation and Ministry for Primary Industries to conduct an updated risk assessment for Maui’s dolphins and accelerate the review of the Maui’s Dolphin Threat Management Plan. Consequently, commercial and recreational set net restrictions were extended slightly to reduce entanglement risk to Maui’s dolphins utilizing the southern part of their distribution, as well as any Hector’s dolphins that disperse north into that area. The results related to the population of Hector’s dolphins in Cloudy Bay provide information that will contribute to the upcoming review of the Hector’s dolphin component of the Threat Management Plan.
All in a DNA’s Work: Conservation Genetics and Monitoring of the New Zealand Endemic Maui’s and Hector’s Dolphins

by
Rebecca Marie Hamner

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Scott Baker was also involved with the development and editing of Chapters 1, 5, and 6.
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DEDICATION

To those who persevere…

…especially when it seems that “the job is never completed.”
1. General Introduction

From the smallest bacterium to the largest blue whale, we are surrounded by an astounding diversity of life. As anthropogenic impacts become more pervasive, leaving no part of Earth left untainted, this vast diversity is dwindling. The current extinction rate of mammals is estimated to be 36 to 78 times the background rate suggested by the fossil record (Regan et al. 2001). Observations like this have led some to suggest that the sixth mass extinction on Earth has already begun (Barnosky et al. 2011).

1.1. Conservation Genetics

The broad field of conservation biology developed in the 1980s as a multidisciplinary response to the recognition of a biodiversity crisis (Groom et al. 2006). The goal of conservation biology is to maintain a vast array of biodiversity in the form of genes, populations, species, habitats, ecosystems, and landscapes, as well as the natural and evolutionary processes that maintain them within a functional ecological setting that is constantly changing (Groom et al. 2006). In order to achieve this goal, conservation biology draws from a range of disciplines, including population genetics, ecology, biogeography, economics, sociology, anthropology, philosophy, etc. Such a diverse range of expertise is necessary to properly inform and attempt to balance the ‘ivory tower’ goal of conserving a maximum amount of diversity with the needs and impacts of an ever-growing population. Genetics plays a fundamental role in conservation biology because it examines the smallest scale of biodiversity, genetic variation, which is the source of biodiversity at higher taxonomic and ecological levels.

In the early 1990s, the sub-discipline of conservation genetics emerged to utilize the tools and concepts of genetics and apply them to problems in conservation biology (Amos and Hoelzel 1992, Hedrick and Miller 1992). Conservation genetics seeks to describe,
monitor and conserve genetic diversity within and among species; elucidate the
demographic and evolutionary mechanisms involved in creating the observed patterns;
and provide a theoretical and empirical framework for guiding conservation management
decisions (Amos and Hoelzel 1992, Hedrick and Miller 1992). Genetic data can provide
answers to questions about both demographic and genetic population parameters that are
critical for informed conservation and management considerations. Such parameters
include, but are not limited to, abundance, effective population size, genetic diversity,
inbreeding, and population connectivity.

1.1.1. Abundance
The first question to be asked regarding a population of interest is usually: how many are
there? The number of individuals that comprise a population is referred to as abundance.
For some purposes, the definition of abundance is restricted to a particular segment of the
population, such as sexually mature individuals (e.g., Luikart et al. 2010). Abundance is
the primary metric underlying the classification of endangered species (e.g. number of
mature animals, decline in total abundance; Baker et al. 2010, IUCN 2012a), and is often
the one used to evaluate the success of management actions. The difficulty and
impracticality of directly counting individuals has lead to the development of a variety of
approaches for estimating abundance by distance sampling or capture-mark-recapture
methods (e.g., Williams et al. 2002). Recently, genotypes have been substituted for
physical tags or natural marks to identify individuals for mark-recapture analysis (Luikart
et al. 2010). Genotype recapture methods are particularly advantageous for the study of
endangered species as they maximize the information gained per effort, which is often
resource-limited. In addition to an abundance estimate, they also allow assessment of
other demographic parameters (e.g., vital rates, sex ratio) and genetic parameters (e.g.,
diversity, population structure, and effective population size; Schwartz et al. 2007).
1.1.2. Effective population size

Effective population size ($N_e$) is the genetic analogue to abundance and a central parameter in population genetics (Wright 1931). It is defined as the size of an ideal population that experiences genetic change at the same rate as the population under consideration (Waples 2002). For endangered species, effective population size is of great interest because it determines the rate at which genetic variability is lost, the rate at which inbreeding increases, and the relative evolutionary importance of selection versus random genetic drift acting on a population (Waples 2002). To guide management decisions, the 50/500 rule was developed to suggest minimum viable effective population sizes (Franklin 1980). It recommends that a minimum $N_e$ of 50 is needed to reduce the likelihood of extinction in the short-term due to harmful effects of inbreeding depression on demography, and a minimum $N_e$ of 500 is needed to prevent the loss of quantitative genetic variation over the long term, to allow future adaptive change and conserve the evolutionary potential of the population. Based on more recent empirical observations, Frankham et al. (2014) recommended an increase to these thresholds, suggesting that an $N_e \geq 100$ is necessary to limit loss of total fitness to $\leq 10\%$ over five generations, and $N_e \geq 1000$ is needed to retain evolutionary potential for fitness in perpetuity. While such thresholds serve as valuable guides for encouraging the prevention of populations from declining below these numbers, some have cautioned that they should not be used as a strict threshold for giving up on species that decline below them as doomed to extinction (Jamieson and Allendorf 2012). Survival could still be possible if the population decline can be halted and the population is allowed to recover at its natural rate of increase to reach a size larger than the recommended thresholds as quickly as possible. In addition to the intrinsic value of estimating $N_e$ for a population, simulations suggest that for small populations, monitoring $N_e$ may be more effective at detecting declines than estimates of abundance ($N_c$), especially if the $N_e/N_c$ ratio is low (Tallmon et al. 2010).
1.1.3. Genetic diversity and bottlenecks

When populations experience a decline in abundance, or a demographic bottleneck, it is often accompanied by a genetic bottleneck, or decline in allelic diversity and heterozygosity (Nei et al. 1975). Bottlenecks are likely to cause a loss of alleles, particularly from highly diverse loci, which can reduce the long-term evolutionary potential of a population. If the population is reduced to a very small size, it will continue to lose alleles and those that remain will be determined by genetic drift, as selection will become ineffective. Heterozygosity is relatively less sensitive to bottlenecks, and affects the ability of a population to evolve in the short-term (Allendorf et al. 2013). The magnitude and duration of a bottleneck, along with life history characteristics of the species of interest, will determine the magnitude of the impact and our ability to significantly detect a bottleneck (Nei et al. 1975, Peery et al. 2012). The greatest losses will occur for populations that are reduced by proportionally larger amounts, held at low numbers for longer durations, and have low potential growth rates.

1.1.4. Inbreeding

When a population declines to a small size with low genetic diversity, extinction risk will increase, in part, due to an increase in inbreeding. The term inbreeding can be defined in several contexts, but in general, describes an increase in homozygosity that results from matings between related individuals (Keller and Waller 2002). The inbreeding coefficient, or probability that two alleles at a locus within an individual will be identical by descent, will increase at a rate of $1/2N_c$ per generation. Therefore in small isolated populations, even if individuals are able to avoid consanguineous matings, genetic drift will still cause the loss of rare alleles and an increase in homozygosity from generation to generation. As homozygosity increases, the expression of accumulated mildly deleterious mutations increases, which can cause a reduction in fitness, referred to as inbreeding depression (Keller and Waller 2002). For example, the inbred remnant population of Florida panthers in the 1990s had reduced reproductive success due to the prevalence of undescended testicles, as well as increased expression of more benign
recessive traits for tail kinks and cowlicks (Hedrick 1995). In such situations, an extinction vortex can result, whereby the effects of inbreeding depression cause a further reduction in population size, which leads to even more inbreeding, and so on until extinction (Gilpin and Soulé 1986). However, as demonstrated by the Florida panther, the introduction of unrelated individuals with novel genetic diversity from a different conspecific population can avert the extinction vortex and lead to genetic rescue (Pimm et al. 2006).

1.1.5. Population connectivity

Population connectivity through dispersal and gene flow among small fragmented populations is critical for maintaining genetic diversity, reducing inbreeding, and ultimately preserving the evolutionary potential and viability of a species. When a population becomes isolated it begins to lose genetic diversity and become more inbred at a rate inversely related to its effective population size \((1/2N_e)\). Natural dispersal by even one individual per generation can increase local genetic diversity and bring about genetic rescue or restoration \((e.g., Vila et al. 2003, Hedrick 2005, Adams et al. 2011)\). Genetic rescue is thought to reduce the risk of inbreeding depression and enhance the chances of long-term species survival (Ingvarsson 2001). However, if gene flow is infrequent and populations have different selection pressures that cause local adaptations to develop, admixture could result in outbreeding depression. This occurs when admixed offspring are less fit because they do not inherit locally adapted alleles or combinations of alleles (Lynch 1991, Marr et al. 2002).

1.2. Genetic Monitoring and Marine Mammals

While assessments of the parameters described above, and many others, at a given point in time are useful for some purposes, genetic monitoring over time provides a powerful tool for conservation and management decisions (Schwartz et al. 2007). By compiling a time-series of assessments, trends in demographic and genetic parameters can be directly
examined, allowing potential causes for population changes to be identified and the success or failure of management actions to be evaluated. Marine mammals present unique challenges to genetic monitoring, which has meant that they have not mirrored the recent exponential increase in genetic monitoring publications for fishes and terrestrial species (Jackson et al. in review). These include logistical and financial challenges to locating individuals, obtaining sufficient genetic sample sizes and spatial coverage, and repeating such assessments long-term to make the monitoring scheme effective. However, as genetic technologies become more efficient and cheaper, and multiple demographic and genetic objectives can be achieved from one set of surveys to collect genetic samples (i.e., genotype recapture abundance and vital rates, and genetic parameters), such studies are becoming more feasible. The information gathered by such studies will be of great benefit to the large number of marine mammals about which little is known, especially the ones that are known to be at risk.

An IUCN review of marine mammals listed 25% as ‘Vulnerable,’ ‘Endangered’ or ‘Critically Endangered’ and 35% as ‘Data Deficient’ (Polidoro et al. 2008). Even point estimates for basic demographic parameters, such as abundance, are unknown for many marine mammals, including those listed in one of the threat categories of the IUCN Red List. For example, at the time of my search (26 Sep 2012), the Red List included 27 cetacean species or subspecies listed as vulnerable \((n = 9)\), endangered \((n = 14)\), or critically endangered \((n = 4)\) (IUCN 2012b). A literature search for estimates of their abundance revealed that two of these taxa did not have estimates at all, and others were partial-range only (representing a minimum abundance), outdated, or imprecise aggregates or approximations (Appendix I).

The risk of extinction for marine mammals is thought to be greater for those that have a slow rate of reproduction, small geographic range, small group size, and coastal or riverine distribution (Davidson et al. 2012). The baiji \((Lipotes vexillifer)\) was characterized by all four of these life history traits and became the first cetacean to go
extinct due to human activity (Turvey et al. 2007). Despite classification as endangered in 1986 and critically endangered in 1996 by the International Union for Conservation of Nature (IUCN 2012b), little effort was made to mitigate the high rate of incidental fisheries-related mortality that drove the decline of the baiji. As a consequence, the last documented baiji sighting was in 2002, and the species is now considered extinct (Turvey et al. 2007, Committee on Taxonomy 2012). Maui’s and Hector’s dolphins are also characterized by these four predictors of extinction and the risk of fishery-related mortality.

1.3. Maui’s and Hector’s Dolphins

The Hector’s dolphin species (*Cephalorhynchus hectori* van Beneden 1881) is endemic to the coastal waters of New Zealand. It is currently classified as two subspecies: *C. h. maui*, referred to as the Maui’s dolphin, and *C. h. hectori*, which retains the common name of Hector’s dolphin (Baker et al. 2002). To avoid confusion, hereafter the common name “Hector’s dolphin” will be used to refer to the subspecies, unless otherwise noted.

Hector’s and Maui’s dolphins are easily distinguished from other dolphin species by their characteristic rounded dorsal fin with convex trailing edge, and striking black, white and gray color pattern (Figure 1.1). They are the smallest member of the family Delphinidae (Perrin et al. 2008), reaching a maximum length of approximately 1.5 meters, with Maui’s dolphins attaining a slightly larger average size than Hector’s dolphins (Slooten and Dawson 1988, Russell 1999, Duignan and Jones 2005). Maui’s dolphins also have a slightly larger rostrum width at half-length, which was one of the morphological characters used for the classification of the subspecies (Baker et al. 2002). However, there are no diagnostic differences that can be used to visually distinguish the two subspecies from each other. The subspecies classification was further supported by evidence of geographic and genetic isolation (Baker et al. 2002), as discussed below.
1.3.1. Distribution and home range

Stranding and sighting records suggest that Maui’s dolphins were once widely distributed along the west coast of the North Island, and possibly parts of the east coast (the subspecies of the east coast records are unknown; Du Fresne 2010). However, over the past 100 years their distribution has contracted to about 300 km along the west coast of the North Island (Dawson et al. 2001), with the majority of recent sightings concentrated in a central distribution of approximately 140 km (Figure 1.2; Appendix III, Oremus et al. 2012). The Hector’s dolphin inhabits the coastal waters of the South Island, where it is unequally distributed among four genetically differentiated populations (Figure 1.2; Hamner et al. 2012; see also Genetic Differentiation).

Both subspecies are usually sighted within a few kilometers of shore (e.g., Dawson and Slooten 1988, Dawson et al. 2004, Oremus et al. 2012), but have been documented ranging out to 35 km (Stone et al. 2005), and exhibit an ‘offshore’ shift during the austral winter (Slooten and Dawson 1988, Bräger et al. 2003, Rayment 2006, Rayment et al. 2010). A more recent inference from sighting records suggests the 100 m depth contour as the limit to their range, as opposed to a particular distance from shore (Dawson et al. 2004, Slooten et al. 2004, Slooten et al. 2006a, Slooten et al. 2006b, Slooten 2007). These dolphins exhibit relatively small home ranges along the coastline. Previous studies of the movements of Hector’s dolphins suggest that individuals are not likely to regularly move across distances larger than approximately 60 km along shore, with only rare movements in excess of 100 km (Bräger et al. 2002, Stone et al. 2005, Rayment et al. 2009).

1.3.2. Genetic differentiation

The small home ranges of these dolphins and the relatively large geographic gaps in their distribution have likely contributed to the genetic differentiation between the two subspecies and among the populations of Hector’s dolphins (Hamner et al. 2012). Since a concerted effort to collect samples began in 1988, the Maui’s dolphin has been
characterized by a single unique mtDNA control region haplotype (G), as compared to the 20 other mtDNA haplotypes described among the Hector’s dolphin populations (Hamner et al. 2012). The complete reproductive isolation of the two subspecies is also supported by strong differentiation at nuclear microsatellite loci (9 loci, $F_{ST} = 0.167$, $P < 0.001$), and clear assignment of individuals by genotype assignment tests (Hamner et al. 2012).

The Hector’s dolphin is comprised of three genetically differentiated regional populations on the east, west and south coasts of the South Island (Figure 1.2; Hamner et al. 2012). Although they show strong genetic differentiation (pairwise mtDNA $F_{ST} = 0.120 – 0.391$, 13-microsatellite $F_{ST} = 0.039 – 0.071$, all $P < 0.001$), low levels of occasional gene flow among the regions were suggested by the identification of five Hector’s dolphins likely to have a migrant father from another regional population (Hamner et al. 2012). Within the East and West Coasts, there appears to be sufficient step-wise gene flow to maintain genetic diversity within the regions; however, the two local South Coast populations (Te Waewae Bay and Toetoe Bay) exhibited a high degree of differentiation (pairwise mtDNA $F_{ST} = 0.136$, 13-microsatellite $F_{ST} = 0.043$, all $P < 0.05$), likely due to the ~100 km gap in distribution between them (Figure 1.2; Hamner et al. 2012).

1.3.3. Life history and vital rates

The life history traits for Hector’s and Maui’s dolphins have been largely inferred from the necropsy of by-caught and beachcast specimens. The maximum age, inferred by counting annual tooth layers, is 19 years for females and 20 years for males (Slooten 1991). However, evidence from living dolphins suggests greater longevity, as two dolphins have been photo-identified over a time span of 21 years (the length of the study; Rayment et al. 2009). Sexual maturity is attained between six and nine years of age for males, and between seven and nine years for females (Slooten 1991). Mating behavior appears to be promiscuous, and has only been sighted in the austral autumn and winter, peaking in early winter (Slooten et al. 1993). Females give birth to a single calf during
the following spring or early summer (Slooten and Dawson 1988). Calves remain with the mother for one to two years, and females have a calving interval of two to three years (Dawson and Slooten 1993).

These life history characteristics mean that Hector’s dolphins, like many other small cetaceans, have a low potential for population growth. Juvenile survivorship is unknown, but adults have an estimated mean survival rate of 89% (Slooten et al. 2000). Their maximum population growth rate is calculated to be 4.9% per year, assuming a 95% non-calf survival rate and optimal population growth parameters (Slooten and Lad 1991). However, Slooten and Lad (1991) reported that a realistic range for the population growth rate is 1.8 - 4.4% and suggest that 2.2% per year is the most likely rate.

1.3.4. Abundance

The Maui’s dolphin currently exists only as a remnant population concentrated along a few hundred kilometers of the central west coast of the North Island (Appendix III, Oremus et al. 2012). Published abundance estimates dating back to 1985 have all been below 140 individuals (Table 1.1), with the most recent conducted in 2004 producing an estimate of 111 (95% CI = 48 – 252; CV = 0.44; Slooten et al. 2006a). This is consistent with a capture-recapture analysis based on genotypes from samples collected between 2001 and 2007, which estimated the population to be N = 69 (95% CI = 38 – 125) at the approximate midpoint of the sample collection in 2003 (Baker et al. 2013).

The overall abundance of Hector’s dolphins is estimated to be 7,270 (95% CL = 5,303 – 9,966; CV = 0.162), unevenly distributed around the South Island (Slooten et al. 2004). The highest concentrations exist on the west coast (N = 5,388; 95% CL = 3,613 – 8,034; CV = 0.206; Slooten et al. 2004), with lower numbers along the east coast (N = 1880; 95% CL = 1,246 – 2,843; CV = 0.213; Dawson et al. 2004) and the lowest numbers inhabiting several bays of the south coast: Te Waewae Bay, N = 259 (95% CL = 185-361; CV = 0.171) in autumn and N = 403 (95% CL = 280-488; CV = 0.121) in summer (New
Interest in studying the population of Hector’s dolphins in Cloudy and Clifford Bays arose in light of a proposal to establish a marine farm in Clifford Bay and the utility of this more abundant population as a contrast to the remnant population of Maui’s dolphins. Aerial line-transect surveys were conducted July 2006 – March 2009 to estimate the abundance of Hector’s dolphins in these bays. Abundance was estimated to be at a maximum in the austral summer with \( N = 951 \) (95% CL = 573 - 1577; CV = 0.263) and a minimum in the spring with \( N = 188 \) (95% CL = 100 - 355; CV = 0.332 (Du Fresne and Mattlin 2009). The large discrepancy between the estimated summer abundance obtained from these aerial surveys and an estimate of 162 (95% CL = 56 - 474; Dawson et al. 2004) obtained by boat surveys conducted December 1999 to February 2000 illustrates the difficulty of estimating the abundance of cetaceans.

### 1.3.5. Threats
Threats to Hector’s and Maui’s dolphins come from a variety of natural and anthropogenic sources including: predation, disease/parasites, inbreeding, stochastic effects on small populations, climate/environmental change, fishing activities, marine farming, commercial tourism, vessel traffic, pollution, mining and oil activities, military activities, coastal development, and research activities. (New Zealand Department of Conservation and Ministry of Fisheries 2007). Between 1921 and October 31, 2013, the New Zealand Department of Conservation (2014) has documented the deaths of 497 Hector’s dolphins and 44 Maui’s dolphins, of which 215 and 7, respectively, showed evidence of some type of human caused mortality, while many others had an unknown or indeterminable cause of death. Mortalities from human-related threats often receive the most attention, as these are the ones that can be managed.
The coastal habitat of Hector’s and Maui’s dolphins coincides with areas utilized by commercial and recreational set net fisheries and inshore trawl fisheries (New Zealand Department of Conservation and Ministry of Fisheries 2007). This overlap has led to significant impacts from fisheries-related mortality, which has been identified as the primary threat to these dolphins (Dawson 1991, Dawson and Slooten 2005, Slooten 2007). Recreational and commercial set netting occurred at low levels in New Zealand from the late 1920s to the early 1970s. Effort dramatically increased around 1970 with the development of monofilament nylon nets and fisheries deregulation, which led to a high rate of incidental set net entanglement of Hector’s and Maui’s dolphins (Dawson 1991, New Zealand Department of Conservation and Ministry of Fisheries 1994, Dawson and Slooten 2005, Slooten 2007).

1.3.6. Conservation actions

The Hector’s dolphin is currently classified as ‘endangered’ by the International Union for the Conservation of Nature (IUCN 2012b) and ‘nationally endangered’ by the New Zealand Threat Classification (Baker et al. 2010), while the Maui’s dolphin is classified as ‘critically endangered’ and ‘nationally critical’ by the respective schemes. Efforts to conserve Hector’s and Maui’s dolphins have been focused on reducing fishery-related mortality, as this was responsible for the majority of documented anthropogenic mortalities (New Zealand Department of Conservation 2014). The Hector’s dolphin population around Banks Peninsula was the first to be given protection in 1988 with the creation of the Banks Peninsula Marine Mammal Sanctuary (Dawson and Slooten 1993). For the Maui’s dolphin, a ban on commercial set nets within 4 NM and trawl nets within 1 NM of the coast from Maunganui Bluff to Pariokariwa Point (Figure 1.2) was announced in 2001, but a legal challenge by fisheries interests meant that it did not take effect until late 2002. In 2008, the ban on commercial and recreational set nets was extended from 4 to 7 NM offshore for this length of coastline. The trawl net ban was also extended to 4 NM for a section between Manukau Harbour and Port Waikato, considered to have the highest density of dolphins, and to 2 NM for the remaining area. The West
Coast North Island Marine Mammal Sanctuary was also declared at this time, which placed restrictions on seabed mining activities and acoustic seismic survey work (New Zealand Department of Conservation and Ministry of Fisheries 2007). For Hector’s dolphins on the South Island’s east coast (from Cape Jackson in the Marlborough Sounds to Slope Point in the Catlins) and south coast (from Slope Point in the Catlins to Sand Hill Point east of Fiordland) commercial and recreational set nets were banned offshore to 4 NM and trawling was banned out to 2 NM. On the South Island’s west coast (from Farewell Spit Lighthouse to Awarua Point north of Fiordland) commercial and recreational set netting and trawling were banned out to 2 NM. The Banks Peninsula Marine Mammal Sanctuary was also expanded at this time and additional marine mammal sanctuaries were created in Cloudy and Clifford Bays, the Catlins Coast, and Te Waewae Bay to restrict fishing activities (New Zealand Department of Conservation and Ministry of Fisheries 2007). Fisheries interests again lodged a legal challenge, delaying enactment of these measures until 2010 (New Zealand Ministry of Fisheries 2010).

Even with the described fishery restrictions, the on-going discovery of beachcast Hector’s and Maui’s dolphins is concerning for the future of these subspecies. The decrease in evidence of entanglement on dolphins found beachcast seems promising, but represents only the small fraction of dolphin carcasses that become beachcast and are recovered after death. On the other hand, the recovery of beachcast dolphins, including pregnant females and neonates that show no signs of entanglement or determinable cause of death (New Zealand Department of Conservation 2014) suggests that inbreeding effects might be starting to cause pregnancy/birth-related complications.

The New Zealand Department of Conservation and Ministry for Primary Industries (which includes the former Ministry of Fisheries) continue to encourage and facilitate research to inform management decisions, particularly for the top four research priorities listed in the 2007 Hector’s and Maui’s Dolphin Threat Management Plan: distribution, abundance, gene flow, and life history characteristics (New Zealand Department of Conservation and Ministry of Fisheries 2007).
1.4. Research Scope and Structure

My PhD research used genetic tools to examine demographic and genetic parameters relevant for conservation considerations regarding Maui’s and Hector’s dolphins, as well as to build upon past genetic baselines for the purpose of long-term genetic monitoring of these subspecies. This first chapter has provided background information to convey the context and motivations for my research.

Chapter 2 is reformatted from the publication: Hamner, R. M., R. Constantine, M. Oremus, M. Stanley, P. Brown and C. S. Baker. 2014. Long-range movement by Hector’s dolphins provides potential genetic enhancement for critically endangered Maui’s dolphin. Marine Mammal Science 30: 139-153. It describes the unexpected discovery of six dolphins on the North Island that belong to the Hector’s dolphin subspecies. This provided the first contemporary evidence of Hector’s and Maui’s dolphins co-occurring in the same area and documented a record dispersal distance observed for the species by at least two of the individuals. Although there was no evidence of interbreeding (gene flow) between the two subspecies at the time of the study, the documentation of Hector’s dolphins naturally dispersing to the North Island provides the potential for increasing the low genetic diversity of the Maui’s dolphin and preserving the species as part of the west coast North Island ecosystem.

Chapter 3 is reformatted from the publication: Hamner, R. M., P. Wade, M. Oremus, M. Stanley, P. Brown, R. Constantine and C. S. Baker. In press. Critically low abundance and limits to human-related mortality for the Maui’s dolphin. Endangered Species Research. It describes the use of genotype recapture analysis to confirm the critically low abundance of the Maui’s dolphin with higher precision than previous estimates made between 1985 and 2004. Additionally, the new abundance estimate was used to update the potential biological removal as a guide to the threshold for human related mortality beyond which recovery is not likely to occur.
Chapter 4, entitled “Comparing estimates of effective population size ($N_e$) and abundance from microsatellite genotyping of Maui’s and Hector’s dolphins”, describes $N_e$ in relation to abundance, and consequent inferences about evolutionary potential and risk of inbreeding depression. Estimates were compared between two Maui’s dolphin datasets from different times, as well as to a more abundant population of its sister subspecies, the Hector’s dolphin in Cloudy Bay. This chapter is intended to be submitted for publication with the following authors: Rebecca M. Hamner, Robin Waples, Rochelle Constantine, Rob Mattlin, and C. Scott Baker.

Chapter 5, entitled “A deep spot in the shallow gene pool: the genetic characteristics of the remnant population of Maui’s dolphins, as compared with Cloudy Bay Hector’s dolphins”, describes the genetic characteristics typically affected by decline and small population size. Genetic diversity (number of alleles and heterozygosity) and the level of inbreeding ($F_{IS}$ and internal relatedness) were compared between the remnant population of Maui’s dolphins and the more abundant population of Hector’s dolphins in Cloudy Bay, as well as between two Maui’s dolphin datasets from different time periods. Tests for bottlenecks were conducted for all populations and simulations were carried out to predict the expected genetic diversity of the Maui’s dolphin under several possible past and future bottleneck scenarios. Additionally, the potential influence of diversifying selection was examined for one microsatellite locus with anomalously high diversity. This chapter is intended to be submitted for publication with the following authors: Rebecca M. Hamner and C. Scott Baker.

Chapter 6, the “General Discussion”, synthesizes the findings and conservation implications of the previous chapters. This chapter includes discussion of management decisions influenced by the results of my work and concludes with future research questions that arose from my findings.
1.4.1. Appendices

Components of my PhD research that were not represented in the above-listed chapters/publications are included as appendices.

Appendix I summarizes a literature review of abundance estimates reported for the 27 cetacean species and subspecies classified as vulnerable (VU), endangered (EN), or critically endangered (CR) by the IUCN Red List.


Appendix V describes tissue samples and datasets that have been archived to facilitate long-term genetic monitoring and future work on Maui’s and Hector’s dolphins.

1.5. References


Table 1.1. Maui’s dolphin abundance (N) estimates for 1985 to 2004 using a variety of methods. Associated 95% confidence limits (CL) and coefficients of variation (CV) are also included, unless not reported (nr) by the source.

<table>
<thead>
<tr>
<th>Method</th>
<th>Applicable year(s)</th>
<th>N</th>
<th>95% CL</th>
<th>CV</th>
<th>Reference</th>
</tr>
</thead>
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<tr>
<td>Boat line-transect</td>
<td>1985</td>
<td>134</td>
<td>nr</td>
<td>nr</td>
<td>Dawson and Slooten 1988</td>
</tr>
<tr>
<td>Computer modeling</td>
<td>1985</td>
<td>140</td>
<td>46 - 280</td>
<td>nr</td>
<td>Martien et al. 1999</td>
</tr>
<tr>
<td>Boat line-transect</td>
<td>1998</td>
<td>80</td>
<td>nr</td>
<td>nr</td>
<td>Russell 1999</td>
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<tr>
<td>Aerial line-transect</td>
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<td>75</td>
<td>48 - 130</td>
<td>0.24</td>
<td>Ferreira and Roberts 2003</td>
</tr>
<tr>
<td>Genotype recapture</td>
<td>2003</td>
<td>69</td>
<td>38 - 125</td>
<td>nr</td>
<td>Baker et al. 2013</td>
</tr>
<tr>
<td>Aerial line-transect</td>
<td>2004</td>
<td>111</td>
<td>48 - 252</td>
<td>0.44</td>
<td>Slooten et al. 2006a</td>
</tr>
</tbody>
</table>

Figure 1.1. The characteristic rounded dorsal fin and coloration of (a) the Maui’s dolphin and (b) the Hector’s dolphin.
Figure 1.2. Distribution of Maui’s and Hector’s dolphins. The black and orange striped section indicates the core distribution of the remnant Maui’s dolphin population. The two populations of Te Waewae Bay and Toetoe Bay make up the South Coast regional population.
2. Long-range movement by Hector’s dolphins provides potential genetic enhancement for critically endangered Maui’s dolphin
2.1. Abstract

For endangered populations with low genetic diversity, low levels of immigration could lead to genetic rescue, reducing the risk of inbreeding depression and enhancing chances of long-term species survival. Our genetic monitoring of Maui’s dolphins revealed the first contemporary dispersal of their sister subspecies, Hector’s dolphin, from New Zealand’s South Island into the Maui’s dolphin distribution along ~300 km of the North Island’s northwest coast. From 2010 to 2012, 44 individuals were sampled within the Maui’s dolphin distribution, 4 of which were genetically identified as Hector’s dolphins (two living females, one dead female, one dead male). We also report two Hector’s dolphins (one dead female neonate, one living male) sampled along the North Island’s southwest coast, outside the presumed range of either subspecies. Together, these records demonstrate long-distance dispersal by Hector’s dolphins (≥400 km) and the possibility of an unsampled Hector’s dolphin population along the southwest coast of the North Island. Although two living Hector’s dolphins were found in association with Maui’s dolphins, there is currently no evidence of interbreeding between the subspecies. These results highlight the value of genetic monitoring for subspecies lacking distinctive physical appearances as such discoveries are not detected by other means, but have important conservation implications.
2.2. Introduction

When a population becomes isolated and loses genetic diversity, natural dispersal by even one individual per generation can increase genetic diversity and bring about genetic rescue (Vila et al. 2003, Adams et al. 2011). Genetic rescue is thought to reduce the risk of inbreeding depression and enhance the chances of long-term species survival (Ingvarsson 2001). For endangered species with natural or anthropogenic limitations to dispersal, human-mediated translocations are sometimes used to maintain or restore genetic diversity (Griffith et al. 1989, Wolf et al. 1996, Benson et al. 2011). For endangered dolphins, however, genetic rescue via natural dispersal has never been documented and human-mediated translocation has never been attempted. Although human-mediated translocation was considered for the baiji (*Lipotes vexillifer*), the species went extinct before implementation of the plan (Wang et al. 2006).

The New Zealand endemic Hector’s dolphin (*Cephalorhynchus hectori* van Beneden 1881) is thought to have declined in distribution and abundance as a result of fisheries-related mortality since the 1970’s (Martien et al. 1999, Slooten and Dawson 2010, Slooten and Davies 2012). This species was classified as two subspecies – the Maui’s dolphin (*C. h. maui*) and the Hector’s dolphin (*C. h. hectori*) – by Baker et al. (2002) and supported by a later review (Perrin et al. 2009). The critically endangered Maui’s dolphin is surviving as a remnant population along ~300 km of the west coast of New Zealand’s North Island, with the core concentration occurring within only 150 km of this distribution (Figure 2.1; Reeves et al. 2008, Baker et al. 2013, Oremus et al. 2012). The more abundant South Island subspecies retains the common name of Hector’s dolphin and is divided into three genetically differentiated regional populations on the east, west and south coasts of the South Island (Hamner et al. 2012). The two subspecies are recognized, in part, based on a diagnostic distinction in mitochondrial (mt) DNA haplotypes (Baker et al. 2002). Since a concerted effort to collect samples began in 1988, the Maui’s dolphin has been characterized by a single unique mtDNA control region haplotype (G), as compared to the 20 mtDNA haplotypes currently found among the Hector’s dolphin subspecies around the South Island (Hamner et al. 2012). The only
potential exceptions were three historical museum samples reportedly collected on the North Island, which had haplotypes otherwise found only in Hector’s dolphins [J in the Bay of Islands c.1870, N in Waikanae in 1967, and J in Oakura in 1988; note: the latter two are corrected from Baker et al. (2002) to match Pichler (2001)]. However, doubts about the reported collection location of one specimen and potential for post-mortem drift of the other two recovered carcasses, as well as evidence that their skeletal measurements were more similar to those of Hector’s dolphins, led Baker et al. (2002) to exclude them from the analyses used to define the two subspecies.

The current genetic isolation of the two subspecies is likely maintained by the relatively large geographic distance between them and the small average home ranges of these dolphins (Hamner et al. 2012). Previous studies on the movements of Hector’s dolphins suggest that individuals are not likely to regularly move across distances larger than approximately 60 km, with only rare movements in excess of 100 km (Bräger et al. 2002, Stone et al. 2005, Rayment et al. 2009). This limited movement is consistent with the limited gene flow observed within the Hector’s dolphin subspecies, among the East Coast, West Coast, and southern Te Waewae Bay and Toetoe Bay populations (Figure 2.1; Hamner et al. 2012).

Genetic monitoring provides a framework for assessing changes in the demographic and genetic status of a species by establishing a baseline genetic assessment from an initial sampling event, followed by the continued collection and analysis of samples over time (Schwartz et al. 2007). Here we report the unexpected natural dispersal of four Hector’s dolphins detected between 2010 and 2012 through the genetic monitoring of the Maui’s dolphin along the northwest coast of the North Island (Oremus et al. 2012). We also report two additional Hector’s dolphins that were sampled in 2005 and 2009 on the southwest coast of the North Island, outside the known distributions of either subspecies. The northward dispersal of Hector’s dolphins into the distribution of the Maui’s dolphin could lead to the genetic enhancement of Maui’s dolphins and promote the preservation of the species as part of the west coast North Island marine ecosystem.
2.3. Materials and Methods

As part of an on-going collaborative program for monitoring the abundance and genetic diversity of Maui’s dolphins, small skin samples were collected via dart biopsy (Krützen et al. 2002) during boat-based surveys conducted from 4 February – 2 March 2010 and from 14 February – 10 March 2011 (Figure 2.1; see Oremus et al. 2012). Additionally, a dart biopsy sample collected from a single dolphin sighted in Wellington Harbour in 2009, and skin samples collected from all Maui’s or Hector’s dolphins recovered as beachcast or entangled carcasses through 25 April 2012 were provided to us by the New Zealand Department of Conservation and archived in the New Zealand Cetacean Tissue Archive at the University of Auckland (Thompson et al. 2013).

All samples were stored in 70% ethanol at -20°C prior to tissue digestion with proteinase K followed by total cellular DNA extraction using a standard phenol:chloroform protocol (Sambrook et al. 1989) as modified for small samples by Baker et al. (1994). We assembled DNA profiles for each sample, including genetic sex identification, mtDNA control region haplotype and 21-locus microsatellite genotypes. Existing DNA profiles previously reported for the Maui’s dolphin baseline samples collected in 2001-2007 (Baker et al. 2013) and for the samples collected in 2010-2011 (Oremus et al. 2012) were built upon to complete the extended DNA profiles described here.

Sex was identified using a multiplexed PCR protocol to amplify fragments of the sry and ZFX/ZFY genes according to Gilson et al. (1998). A fragment of approximately 700 bp from the 5’ end of the maternally-inherited mtDNA control region was amplified and sequenced according to Hamner et al. (2012). Sequences were aligned and edited using Geneious Pro v5.5.2 (BioMatters). Haplotypes were initially assigned based on the 360 bp reference sequences of the 22 haplotypes previously identified for Hector’s and Maui’s dolphins (Pichler et al. 1998, Pichler and Baker 2000, Pichler 2002, Hamner et al. 2012), however several of these haplotypes were further resolved based on alignment with longer 576 bp sequences.
All samples were genotyped for 21 microsatellite loci using published cetacean primers (Table 2.1). For the ‘SGUI’ loci and TtruGT48, each 10 µL PCR reaction contained 1x PCR II buffer, 2.5 mM MgCl₂, 0.04 µM of the forward primer with M13 tag, 0.4 µM reverse primer, 0.4 µM fluorescent label with M13 tag, 0.2 mM dNTP, 20 mg/mL bovine serum albumin (BSA), 0.25 units Platinum Taq (Invitrogen) and 10 – 20 ng/µL DNA template, and were amplified using the thermocycling profile of Cunha and Watts (2007) with modifications to the annealing temperature specified in Table 2.1. For all other loci, each 10 µL PCR reaction contained 1x PCR II buffer, 2.5 mM MgCl₂, 0.4 µM each primer, 0.2 mM dNTP, 0.125 units Platinum Taq (Invitrogen) and 10 – 20 ng/µL DNA template, and were amplified using the following thermocycling profile: 93°C for 2 min; (92°C for 30s, Tₐ for 45s, 72°C for 50s) x 15; (89°C for 30s, Tₐ for 45s, 72°C for 50s) x 20; 72°C for 3 min, with the annealing temperatures (Tₐ) stated in Table 2.1. Products were run on an ABI 3130XL DNA Analyzer and allele peaks were binned and visually verified using GENEMAPPER v.3.7 (Applied Biosystems). To minimize genotyping error, each amplification and sizing run included a negative control to detect contamination and 10 internal control samples to ensure comparable allele sizing across all runs and to estimate genotyping error. A genotyping error rate was calculated by dividing the number of incongruent allele calls by the total number of alleles compared for the samples that were genotyped twice (Bonin et al. 2004).

Genotypes were compared to identify replicate samples of the same individual using CERVUS v. 3.0 (Kalinowski et al. 2007). The probability of identity (P_ID) and probability of identity for siblings (P_IDsib) for each locus and across all loci were calculated in GenAlEx v. 6.1 (Peakall and Smouse 2006). To avoid false exclusion, initial matching allowed for up to five mismatching loci, and we examined each of these ‘relaxed matches’ for potential allelic dropout or processing error, and repeated them as needed for confirmation. Sex and mtDNA haplotypes were subsequently compared to support our confidence in correctly identifying replicate samples. After review and replication for correction or confirmation of the ‘relaxed matches’, we accepted samples with matching genotypes as re-samples of the same individual.
For individuals found to have Hector’s dolphin haplotypes (“putative Hector’s dolphins”), as opposed to the characteristic G of the Maui’s dolphin (see Results), the subspecies was confirmed and populations of origin were identified using the Bayesian assignment procedures in the programs Structure v2.3.2 (Pritchard et al. 2000, Pritchard et al. 2010) and GeneClass2 v2.2.2 (Piry et al. 2004). For this, we used a reference data set of genotypes from 10 microsatellite loci in linkage equilibrium for Maui’s dolphins ($n = 87$ individuals) and Hector’s dolphins ($n = 176$ individuals) from across the three regional populations (Hamner et al. 2012). Although several loci showed slight departures from Hardy-Weinberg equilibrium (Hamner et al. 2012), none were significant across all populations. Simulations by Cornuet et al. (1999) suggest that such slight departures from Hardy-Weinberg equilibrium are not likely to influence the result of assignment tests. In Structure, no population information was included for the putative Hector’s dolphins and the “UsePopInfo” option assuming no admixture and correlated allele frequencies was applied to the reference samples to run $10^6$ Markov Chain Monte Carlo (MCMC) replicates following a burn-in of $10^5$ for $K = 4$ populations. A membership coefficient ($q \geq 0.900$) was used as the threshold for confidently identifying the population of origin. This threshold has been accepted as sufficient evidence for prosecution in wildlife poaching cases (i.e., Lorenzini et al. 2011), and is considered more appropriate for management cases given the lower rate of false exclusion of the true identity than the more stringent $q_i = 0.999$ threshold required by other wildlife forensic cases (Manel et al. 2002, Millions and Swanson 2006). In GeneClass2, the Bayesian method of Rannala and Mountain (1997) was implemented to assign the putative Hector’s dolphins to the reference data set described above, using an alpha of 0.01 as evidence of origin. Additionally, Paetkau et al.’s (2004) permutation procedure was implemented with 1,000 simulated individuals and a threshold of $P < 0.01$ to exclude populations as an individual’s origin, as is used in other wildlife applications (Berry and Kirkwood 2010, Drewry et al. 2012).
2.4. Results

A total of 76 samples were collected within the Maui’s dolphin distribution on the northwest coast of the North Island between 2010 and 2012. Of these, 73 were collected from living dolphins during the 2010 and 2011 surveys (Oremus et al. 2012), and 3 were provided to us from recovered dolphin carcasses: Chem10NZ06 collected on 20 November 2010 floating off Raglan, Che11NZ06 collected on 26 October 2011 at Clark’s Beach in Manukau Harbour, and Che12NZ02 collected on 25 April 2012 at Opunake, Taranaki. Our work also considers two dolphins sampled outside of the known distributions of either the Hector’s or Maui’s dolphin subspecies: a neonate found just before death on Peka Peka Beach in 2005 (Che05NZ20) and a single dolphin biopsy sampled during a rare sighting in Wellington Harbour in 2009 (Che09WH01; Figure 2.1).

The DNA profiles identified 46 individual dolphins from the 78 samples described above, with a combined microsatellite $P_{(ID)} = 3.7 \times 10^{-8}$ and $P_{(ID)sib} = 3.1 \times 10^{-4}$ (Table 2.1). No contamination was detected by the negative controls, and a genotyping error rate due to allelic dropout was estimated to be 0.4% based on the repeated genotyping of the 10 control samples (252 alleles). However, the error rate in the final data set is likely to be lower than this, as genotypes of ‘relaxed matches’ were also replicated to either correct allelic dropout or confirm the genotype.

The mtDNA control region sequence of 40 individuals matched the G haplotype that has been diagnostic of the Maui’s dolphin population since the collection of contemporary samples began in 1988 (Pichler and Baker 2000). However, four individuals sampled within the Maui’s dolphin distribution (CheNI10-03, CheNI10-24, Che11NZ06, Che12NZ02) and the two sampled on the southwest coast of the North Island (Che05NZ20, Che09WH01) represented haplotypes found only in Hector’s dolphins: C, H, I, and J (360 bp; Figure 2.1; Table 2.S1), and were considered putative Hector’s dolphins. These are the four most common Hector’s dolphin haplotypes (Hamner et al. 2012), which have now been resolved into three to four sub-types each when using longer 576 bp sequences (unpublished data). Based on these longer sequences, the six dolphins
of interest each have a different haplotype: CheNI10-03, Ib; CheNI10-24, Jb; Che11NZ06, Cb1; Che12NZ02, Hb; Che05NZ20, Ia; and Che09WH01, Ca; GenBank Accessions: KC492580-KC492585). These six haplotypes differ from the G haplotype (also extended to 576 bp; GenBank Accession: KC492586) at two to six sites each. However, as not all samples in the reference data set of Hector’s dolphin haplotypes have the longer sequences, we are unable to examine their relative frequencies in the different Hector’s dolphin populations at this time.

To confirm the subspecies and likely population of origin, the genotypes of the putative Hector’s dolphins were compared to baseline samples described by Hamner et al. (2012). The Structure analysis clearly assigned the six putative Hector’s dolphins to the Hector’s dolphin subspecies, while all other samples collected on the North Island clearly assigned to the Maui’s dolphin (Figure 2.2). Two females sampled alive within the Maui’s dolphin distribution assigned strongly to the population of Hector’s dolphins on the west coast of the South Island (CheNI10-03 q = 0.9790, CheNI10-24 q = 0.9783; Figure 2.2), however, the other four dolphins showed ambiguous assignment to the Hector’s dolphin populations (highest q ≤ 0.6; Figure 2.2). The GeneClass2 analysis further supported the evidence that these six individuals were Hector’s dolphins by excluding the Maui’s dolphin as the population of origin for each of them with high certainty (P ≤ 0.003; Table 2.2). However, the only Hector’s dolphin population that could be excluded as a source was the South Coast for one of the dolphins, Che12NZ02 (P = 0.002). As in the Structure results, GeneClass2 assigned CheNI10-03 and CheNI10-24 to the West Coast South Island with high likelihoods (Table 2.2). GeneClass2 also provided high assignment likelihoods for Che12NZ02 to the West Coast South Island, and for Che09WH01 and Che11NZ06 to the East Coast South Island population, although they did not exceed the high confidence threshold of 0.01 (Table 2.2). Again similar to the Structure results, Che05NZ20 showed a more ambiguous assignment among the Hector’s dolphin populations with a moderate likelihood of 0.6189 to the West Coast, followed by 0.2353 to the East Coast.
2.5. Discussion

Our findings demonstrate the fundamental concept of genetic monitoring – observing changes in demographic and genetic parameters over time. The genetic monitoring of the Maui’s dolphin resulted in the unexpected discovery of four Hector’s dolphins within the Maui’s dolphin distribution on the northwest coast of the North Island between 2010 and 2012. The presence of these Hector’s dolphins would not have been evident without the extensive baseline of genetic diversity initiated by Pichler (2002) and updated by Hamner et al. (2012) to include individuals sampled between 1988 and 2007. This reference sample set was intentionally time-limited so as to minimize the potential for generational changeover, assuming an estimated 20-year maximum lifespan of Hector’s and Maui’s dolphins (Slooten and Lad 1991), while maximizing the number of contemporary samples across the distribution of the species.

In light of the unexpected discovery of the Hector’s dolphins among Maui’s dolphins, we re-examined genotypes of two Hector’s dolphins sampled on the southwest coast of the North Island in 2005 and 2009. These dolphins sampled at Peka Peka Beach (Che05NZ20) and Wellington Harbour (Che09WH01), were found between the distributions of the two subspecies. Although the sample from Peka Peka Beach (Che05NZ20) was collected in 2005, within the 1988-2007 time period used for the baseline, it was excluded from the genetic baseline as an outlier, given that it was a neonate found beachcast in an area extralimital to the known distribution of either subspecies. However when considered together, the six Hector’s dolphins sampled on the North Island pose several non-exclusive scenarios: (A) several independent events occurred where one or more dolphins dispersed from known population(s) on the South Island to the North Island; (B) a single stochastic event occurred, where several Hector’s dolphins dispersed together as a group from a known population on the South Island to the North Island; or (C) a small population of previously unsampled Hector’s dolphins exists along the southern North Island or northern South Island, several of which dispersed into the Maui’s dolphin distribution. We consider a combination of either A and C or B and C to be most consistent with the results of the population assignment analyses.
The discovery of these Hector’s dolphins on the North Island calls for reconsideration of three historical samples described by Baker et al. (2002). These three samples were reportedly collected on the North Island, but did not have the characteristic G haplotype of the Maui’s dolphin. Baker et al. (2002) excluded them from the analyses used to classify the subspecies due to doubts about the actual collection location of one specimen and the potential for post-mortem drift of the two that were found beachcast in advanced states of decomposition. Unfortunately, we have no additional information from these bone and tooth samples to support or refute the provenance of these dolphins or to confirm their subspecies, so cannot determine if they represent historical mtDNA diversity that has been lost from the Maui’s dolphin or if they were in fact migrant Hector’s dolphins.

In any case, the dispersal of Hector’s dolphins into the distribution of the Maui’s dolphin is not likely to have been a frequent occurrence. Using a binomial distribution probability function (Swofford and Berlocher 1987), the chance of detecting a Hector’s dolphin haplotype in the baseline of 96 Maui’s dolphin samples collected from 1988 to 2007 (Hamner et al. 2012) is 93.3% for a Hector’s dolphin haplotype at a frequency of 5%, and 61.9% for a Hector’s dolphin haplotype at a frequency of 1%. More importantly, no genetic admixture between Hector’s and Maui’s dolphins has been detected in any of the 269 individuals from both subspecies that were sampled and genotyped between 1988 and 2012 (Hamner et al. 2012; current work). Furthermore, the BayesAss analysis presented by Hamner et al. (2012), estimated negligible migration rates between the two subspecies, ranging from 0.006 to 0.014 dolphins per generation.

Our findings are the first contemporary evidence of Hector’s and Maui’s dolphins co-occurring in the same area. Although four Hector’s dolphins have now been documented within the geographic range of the Maui’s dolphin, it is premature to raise concerns about the validity of the subspecies. To date, we have not detected evidence of interbreeding between the Hector’s and Maui’s dolphins, and there are no examples from captivity to
assess this potential. The number of documented dispersal events at this time is low. However, if further dispersal of Hector’s dolphins occurs and the subspecies are shown to interbreed, it could lead to a loss of the genetic and morphological distinctiveness that was used to support their classification as subspecies (Reeves et al. 2004, Perrin et al. 2009).

The minimum distance of 400 km required for Hector’s dolphins to travel from the West Coast South Island population to the central northwest coast of the North Island was surprising given previous observations of restricted home ranges. Despite over 25 years of research on Hector’s dolphins, the maximum distance of dispersal observed was just over 100 km and most observed movements have been within a home range of 30-60 km (Bräger et al. 2002, Rayment et al. 2009). The deep water of Cook Strait was thought to deter these dolphins from moving between the North and South Islands, consistent with most observations of Hector’s dolphins occurring in depths less than 39 m (Bräger et al. 2003, Rayment et al. 2011) and the rarity of sightings in the Fiordland area where depths can exceed 300 m (Cawthorn 1988). However, our identification of two Hector’s dolphins from the West Coast South Island confirm that movements between the islands do occasionally occur, even if it is not known whether the dolphins are crossing the deeper waters at the narrowest point of Cook Strait or perhaps following an offshore corridor of shallower water to the northwest.

The ambiguous assignment of four dolphins to the Hector’s dolphin populations, suggests the potential for a previously unsampled population of Hector’s dolphins that is not included in our baseline reference data, or perhaps an area of interbreeding between the East and West Coast Hector’s dolphin populations. Therefore, the potential for a small and elusive resident population of Hector’s dolphins along the southern part of the North Island, outside the current range of the Maui’s dolphin, or along the northern part of the South Island between the East and West Coast populations of Hector’s dolphins should be investigated.
The protection of habitat and removal of anthropogenic threats are crucial if the Maui’s dolphin is to survive (Currey et al. 2012). The New Zealand government has recognized this by establishing the West Coast North Island Marine Mammal Sanctuary and placing restrictions on seabed mining, acoustic seismic surveys, and fishing activities (New Zealand Department of Conservation 2008, New Zealand Ministry of Fisheries 2012). However, with the known distance of individual movement greatly increased to at least 400 km and the confirmation that these dolphins will at least occasionally disperse from the South Island to North Island, there is the possibility that genetic exchange between the subspecies will also benefit the Maui’s dolphin and promote the survival of the species on the west coast of the North Island. If protected corridors connecting the Maui’s dolphin on the North Island and Hector’s dolphin populations on the South Island are not maintained, then such natural dispersal events are less likely to occur.

Rare natural dispersal events similar to the one described here for Hector’s dolphins have been beneficial for improving the genetic diversity and fitness of wolves in Scandinavia (Vila et al. 2003) and Isle Royal National Park (Adams et al. 2011), and perhaps other cases overlooked by a narrow definition of genetic rescue (Hedrick et al. 2011). The genetic rescue of the Isle Royale wolves is thought to be the cause for a slight increase in the population at a time when space and prey were limiting (Adams et al. 2011). Unfortunately, in this case the benefits seem to have been short-lived due to deteriorating environmental conditions and recent stochastic events, which reduced the population to 14 males and 2 females in 2011 (Vucetich et al. 2012). This illustrates the importance of continued monitoring and the need to mitigate all known threats to a population if its chances for surviving stochastic events are to be maximized.

Although the Hector’s dolphin migrants have the potential to enhance the genetic diversity of the Maui’s dolphin, there is also the potential for outbreeding depression to occur if the Maui’s dolphin has undergone selection or specialization making it better adapted to its North Island habitat. Outbreeding depression occurs when ‘hybrid’ offspring do not inherit local adaptations, causing them to be less fit than individuals.
whose parents originate from the same locally adapted population. Although difficult to document in wild populations, this was observed when migrants naturally entered the otherwise isolated song sparrow population on Mandarte Island (Marr et al. 2002). The possibility of local adaptations and outbreeding depression for Hector’s and Maui’s dolphins could be assessed by applying a genomic approach to assess functional genetic divergence between the two subspecies (Allendorf et al. 2010).

Our findings highlight the value of genetic monitoring, particularly for cryptic subspecies or populations, as such discoveries cannot be made by other means, but have important conservation implications. During the time period of our study, one additional dolphin mortality was reported by a commercial fisherman who found it entangled in his set net off Cape Egmont in January 2012 (New Zealand Department of Conservation 2012). Unfortunately, no sample was taken for genetic analysis to confirm the subspecies before the fisherman followed the protocol in place at the time and returned the carcass to the sea.

Only time and continued genetic monitoring will reveal if the living Hector’s dolphin migrants remain permanent North Island residents and if they are successful at contributing to the diminished gene pool of the Maui’s dolphin. Available evidence suggests that the dispersal may be permanent, as CheNI10-24 was sampled in both 2010 and 2011 (Oremus et al. 2012; Table 2.S1). If the female migrants breed with Maui’s dolphins, their relative breeding success can be tracked by monitoring the frequencies of their distinctive maternally-inherited mtDNA haplotypes. Additionally, biparentally-inherited microsatellite genotypes can be used to detect potential evidence of admixture between the subspecies and genetic rescue of the Maui’s dolphin.
2.6. Acknowledgments

Our research was funded by the New Zealand Department of Conservation (DOC), as well as a Mamie Markham Research Award, Ted Thorgaard Student Research Awards, Oregon Lottery Scholarships, and Oregon State University Laurels Scholarships to RMH. Many thanks to everyone at DOC who assisted in the field and with sampling of carcasses, especially C. Duffy, G. Hickman, K. Hilllock, C. Lilley, K. MacLeod, and B. Williams; to N. Gibbs for collecting the Wellington Harbour biopsy sample; to those involved with the current and baseline labwork: A. Alexander, E. Carroll, D. Heimeier, S. Lavery, F. Pichler, K. Russell, D. Steel, K. Thompson and M. Vant; and to V. Ward for the Hector’s dolphin drawing. We are grateful for support from local iwi and DOC Area Offices and Conservancies. We also thank M. Schwartz and three anonymous reviewers for comments to improve the manuscript. Biopsy samples were collected under permit RNW/HO/2009/03 issued to CSB from DOC and the protocol AEC/02/2008/R658 approved by the University of Auckland Animal Ethics Committee.

2.7. References


Table 2.1. Microsatellite loci genotyped for the individual and subspecies identification of Maui's and Hector's dolphins sampled on the northwest coast of New Zealand’s North Island between 2010 and 2012 ($n = 76$ samples, representing 44 individuals), and on the southwest coast of the North Island in 2005 and 2009 ($n = 2$ samples, representing 2 individuals). *Ten loci were also genotyped previously for Hector's dolphins sampled on the South Island (Hamner et al. 2012) and used for subspecies assignment.

<table>
<thead>
<tr>
<th>Locus</th>
<th>F primer label</th>
<th>$T_A$ ($°C$)</th>
<th>$n$ samples (individuals)</th>
<th># alleles</th>
<th>$P_{(ID)}$</th>
<th>$P_{(ID)_{sib}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>415/416$^{\text{a}}$</td>
<td>HEX</td>
<td>45</td>
<td>75 (43)</td>
<td>2</td>
<td>0.464</td>
<td>0.680</td>
</tr>
<tr>
<td>EV1$^{\text{e}}$</td>
<td>HEX</td>
<td>45</td>
<td>76 (44)</td>
<td>1</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>EV14$^{\text{e}}$</td>
<td>VIC</td>
<td>60</td>
<td>78 (46)</td>
<td>4</td>
<td>0.304</td>
<td>0.581</td>
</tr>
<tr>
<td>EV37$^{\text{e}}$</td>
<td>HEX</td>
<td>45</td>
<td>70 (41)</td>
<td>5</td>
<td>0.440</td>
<td>0.675</td>
</tr>
<tr>
<td>EV94$^{\text{e}}$</td>
<td>FAM</td>
<td>55</td>
<td>78 (46)</td>
<td>6</td>
<td>0.263</td>
<td>0.552</td>
</tr>
<tr>
<td>GT211$^{\text{f}}$</td>
<td>FAM</td>
<td>50</td>
<td>76 (44)</td>
<td>4</td>
<td>0.229</td>
<td>0.504</td>
</tr>
<tr>
<td>GT23$^{\text{f}}$</td>
<td>VIC</td>
<td>55</td>
<td>78 (46)</td>
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<td>0.373</td>
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<td>GT575$^{\text{f}}$</td>
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<td>78 (46)</td>
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<td>0.906</td>
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<td>KWM12a$^{\text{g}}$</td>
<td>VIC</td>
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<td>77 (45)</td>
<td>11</td>
<td>0.214</td>
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<tr>
<td>KWM9b$^{\text{h}}$</td>
<td>FAM</td>
<td>50</td>
<td>77 (45)</td>
<td>6</td>
<td>0.172</td>
<td>0.459</td>
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<tr>
<td>MK6$^{i}$</td>
<td>NED</td>
<td>50</td>
<td>74 (42)</td>
<td>3</td>
<td>0.809</td>
<td>0.901</td>
</tr>
<tr>
<td>PPHO110$^{j}$</td>
<td>FAM</td>
<td>50</td>
<td>77 (45)</td>
<td>4</td>
<td>0.274</td>
<td>0.545</td>
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<tr>
<td>PPHO130$^{j}$</td>
<td>NED</td>
<td>55</td>
<td>78 (46)</td>
<td>3</td>
<td>0.816</td>
<td>0.905</td>
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<tr>
<td>PPHO142$^{j}$</td>
<td>NED</td>
<td>55</td>
<td>78 (46)</td>
<td>2</td>
<td>0.393</td>
<td>0.615</td>
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<tr>
<td>SGU106$^{k}$</td>
<td>M13-VIC</td>
<td>57</td>
<td>72 (41)</td>
<td>4</td>
<td>0.895</td>
<td>0.946</td>
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<tr>
<td>SGU107$^{k}$</td>
<td>M13-NED</td>
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<td>76 (44)</td>
<td>3</td>
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<td>SGU116$^{k}$</td>
<td>M13-VIC</td>
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<td>2</td>
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<td>0.664</td>
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<tr>
<td>SGU117$^{k}$</td>
<td>M13-NED</td>
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<td>75 (43)</td>
<td>3</td>
<td>0.372</td>
<td>0.597</td>
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<tr>
<td>TexVet$^{l}$</td>
<td>FAM</td>
<td>50</td>
<td>76 (44)</td>
<td>2</td>
<td>0.925</td>
<td>0.962</td>
</tr>
<tr>
<td>TtruGT48$^{m}$</td>
<td>M13-VIC</td>
<td>55</td>
<td>76 (44)</td>
<td>3</td>
<td>0.493</td>
<td>0.710</td>
</tr>
</tbody>
</table>

| Overall         | 78 (46)        | 3.7 x 10$^{-8}$ | 3.1 x 10$^{-4}$ |

$^{\text{a}}$ $T_A =$ annealing temperature  
$^{\text{b}}$ $P_{\text{ID}} =$ probability of identity  
$^{\text{c}}$ $P_{\text{ID} \text{sib}} =$ probability of identity for siblings  
$^{\text{d}}$ Schlotterer et al. 1991  
$^{\text{e}}$ Valsecchi and Amos 1996  
$^{\text{f}}$ Bérubé et al. 2000  
$^{\text{g}}$ Hoelzel et al. 1998  
$^{\text{h}}$ Hoelzel et al. 2002  
$^{\text{i}}$ Krützen et al. 2001  
$^{\text{j}}$ Rosel et al. 1999  
$^{\text{k}}$ Cunha and Watts 2007  
$^{\text{l}}$ Rooney et al. 1999  
$^{\text{m}}$ Caldwell et al. 2002; forward primer modified to include an M13 tag
Table 2.2. Likelihood of individuals originating in the Maui’s dolphin or East Coast, West Coast or South Coast Hector’s dolphin population calculated using Rannala and Mountain’s (1997) assignment method (highest likelihood in bold) and the probability of exclusion ($P < 0.01$, shaded gray) using Paetkau et al.’s (2004) permutation procedure in `GeneClass2` based on 10-locus microsatellite genotypes and the reference data set of Hamner et al. (2012).

<table>
<thead>
<tr>
<th>Individual</th>
<th>Maui’s dolphin Likelihood</th>
<th>Hector’s dolphin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>East Coast</td>
<td>West Coast</td>
</tr>
<tr>
<td></td>
<td>Likelihood</td>
<td>$P$</td>
<td>Likelihood</td>
</tr>
<tr>
<td>CheNI10-03</td>
<td>0.0000</td>
<td>&lt;0.0001</td>
<td>0.0015</td>
</tr>
<tr>
<td>CheNI10-24</td>
<td>0.0000</td>
<td>&lt;0.0001</td>
<td>0.0000</td>
</tr>
<tr>
<td>Che11NZ06</td>
<td>0.0000</td>
<td>&lt;0.0001</td>
<td><strong>0.9862</strong></td>
</tr>
<tr>
<td>Che12NZ02</td>
<td>0.0000</td>
<td>&lt;0.0001</td>
<td>0.0610</td>
</tr>
<tr>
<td>Che05NZ20</td>
<td>0.0000</td>
<td>&lt;0.0001</td>
<td>0.2353</td>
</tr>
<tr>
<td>Che09WH01</td>
<td>0.0007</td>
<td>0.0030</td>
<td><strong>0.9754</strong></td>
</tr>
</tbody>
</table>
Figure 2.1. Mitochondrial control region haplotypes (360 bp) sampled from the coastal distributions of the Maui’s dolphin and three regional populations of Hector’s dolphins between 1988 and 2012 (Hamner et al. 2012; current work). Haplotypes based on 576 bp sequences are indicated next to the icons for the six Hector’s dolphins found on the North Island, but are not available for all samples in the reference data set at this time.
Figure 2.2. Identification of six Hector’s dolphins using a Structure v.2.3.2 analysis of 10-locus microsatellite genotypes and a reference data set of Maui’s dolphins ($n = 87$) and Hector’s dolphins from the East Coast ($n = 93$), West Coast ($n = 51$), and South Coast ($n = 32$) South Island populations (Hamner et al. 2012). Four of the Hector’s dolphins (CheNI10-03, CheNI10-24, Che11NZ06, Che12NZ02) were sampled within the Maui’s dolphin distribution on the northwest coast of the North Island and two (Che05NZ20, Che09WH01) were sampled between the distributions of either subspecies on the southwest coast of the North Island.
Table 2.S1. Four Hector's dolphins (*Cephalorhynchus hectori hectori*) sampled within the distribution of the Maui's dolphin (*C. h. maui*) on the northwest coast of New Zealand's North Island and *two* Hector's dolphins sampled on the southwest coast of the North Island, outside the previously known distribution of either subspecies.

<table>
<thead>
<tr>
<th>Individual ID</th>
<th>DOC Code</th>
<th>Date Sampled</th>
<th>Location</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Sampled Alive or Dead</th>
<th>Age Class</th>
<th>Sex</th>
<th>mtDNA haplotype (576bp)</th>
<th>GenBank Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>Che05NZ20*</td>
<td>H108/05</td>
<td>2005</td>
<td>Peka Peka Beach</td>
<td></td>
<td></td>
<td>dead</td>
<td>neonate</td>
<td>F</td>
<td>Ia</td>
<td>KC492583</td>
</tr>
<tr>
<td>Che09WH01*</td>
<td>n/a</td>
<td>31-Mar-09</td>
<td>Evans Bay, Wellington Harbour</td>
<td></td>
<td></td>
<td>alive</td>
<td>≥ 1 year</td>
<td>M</td>
<td>Ca</td>
<td>KC492580</td>
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<tr>
<td>CheNI10-03</td>
<td>n/a</td>
<td>5-Feb-10</td>
<td>South of Manukau Harbour</td>
<td>-37.173500</td>
<td>174.578778</td>
<td>alive</td>
<td>≥ 1 year</td>
<td>F</td>
<td>Ib</td>
<td>KC492584</td>
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<tr>
<td>CheNI10-24</td>
<td>n/a</td>
<td>11-Feb-10</td>
<td>Waikato River mouth</td>
<td>-37.360233</td>
<td>174.685983</td>
<td>alive</td>
<td>≥ 1 year</td>
<td>F</td>
<td>Jb</td>
<td>KC492585</td>
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<td></td>
<td></td>
<td>24-Feb-10</td>
<td>South of Waikato River mouth</td>
<td>-37.483067</td>
<td>174.721283</td>
<td>alive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15-Feb-11</td>
<td>South of Manukau Harbour</td>
<td>-37.163950</td>
<td>174.579717</td>
<td>alive</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>18-Feb-11</td>
<td>South of Manukau Harbour</td>
<td>-37.225767</td>
<td>174.611600</td>
<td>alive</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Che11NZ06</td>
<td>H211/11</td>
<td>26-Oct-11</td>
<td>Clark's Beach, Manukau Harbour</td>
<td></td>
<td></td>
<td>dead</td>
<td>≥ 1 year</td>
<td>F</td>
<td>Cb1</td>
<td>KC492581</td>
</tr>
<tr>
<td>Che12NZ02</td>
<td>H221/12</td>
<td>25-Apr-12</td>
<td>Opunake, Taranaki</td>
<td></td>
<td></td>
<td>dead</td>
<td>≥ 1 year</td>
<td>M</td>
<td>Hb</td>
<td>KC492582</td>
</tr>
</tbody>
</table>
3. Critically low abundance and limits to human-related mortality for the Maui’s dolphin

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Nordbünte 23 (+5, 28, 30)
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In press
3.1. Abstract

The New Zealand endemic Maui’s dolphin (*Cephalorhynchus hectori maui*) is characterized by several life history traits thought to be important predictors of extinction risk in marine mammals, including a slow rate of reproduction, small geographic range, small group size, and coastal distribution. We continued the genetic monitoring of the remnant population of Maui’s dolphins using DNA profiles to identify 39 individuals from 73 skin biopsy samples collected during dedicated boat surveys in the austral summers of 2010 and 2011. Using a two-sample, closed-population model with the genotype recapture records, we estimated the current abundance to be $N = 55$ (95% CL = 48, 69, CV = 0.15) individuals approximately age 1+. The endangered species potential biological removal that would permit the recovery of the Maui’s dolphin was calculated to be one dolphin every 10 to 23 years. Despite this, the Maui’s dolphin is not necessarily doomed to extinction. It appears to be maintaining an equal sex ratio and connectivity within its remnant range, and has the potential for rescue by interbreeding with Hector’s dolphin migrants.
3.2. Introduction

The risk of extinction for marine mammals is thought to be greater for those that have a slow rate of reproduction, small geographic range, small group size, and coastal or riverine distribution (Davidson et al. 2012). The baiji (*Lipotes vexillifer*) was characterized by all four of these life history traits and became the first cetacean to go extinct due to human activity (Turvey et al. 2007). Despite classification as endangered in 1986 and critically endangered in 1996 by the International Union for Conservation of Nature (IUCN 2012), little effort was made to mitigate the high rate of incidental fisheries-related mortality that drove the decline of the baiji. As a consequence, the last documented baiji sighting was in 2002, and the species is now considered extinct (Turvey et al. 2007, Committee on Taxonomy 2012).

The Maui’s dolphin (*Cephalorhynchus hectori maui*) is also characterized by these four predictors of extinction and the risk of fisheries-related mortality. The Maui’s dolphin is one of two subspecies of the New Zealand endemic Hector’s dolphin species (*C. hectori*). It is classified as critically endangered by the IUCN (Reeves et al. 2008) and nationally critical under the New Zealand Threat Classification System (Baker et al. 2010). Stranding and sighting records suggest that Maui’s dolphins were once widely distributed along the west coast of the North Island, and possibly parts of the east coast (the subspecies of the east coast records are unknown; Du Fresne 2010). Their current distribution has contracted to about 300 km along the west coast of the North Island, with the majority of sightings concentrated in a central distribution of approximately 140 km (Oremus et al. 2012). Population dynamic models suggested a substantial decline in the abundance of Maui’s dolphins since the advent of nylon monofilament set nets in the late 1960s (Martien et al. 1999, Slooten et al. 2000). In 2001, the New Zealand government proposed fishing restrictions to reduce entanglement, and has since implemented several sets of restrictions within the Maui’s dolphin distribution (summarized by Baker et al. 2013). The West Coast North Island Marine Mammal Sanctuary was also established to restrict seismic surveys and mining activities (New Zealand Department of Conservation 2008).
Monitoring abundance is essential for evaluating the status of Maui’s dolphins and strategies for conservation management. Estimates of abundance calculated from boat and aerial line-transect surveys since 1985 ranged from 75 to 140 (Table 3.1). More recently, Baker et al. (2013) used genotype recapture analysis to establish a census of known individuals and estimate an abundance of \( N = 69 \) (95% CL = 38, 125) for the midpoint of the study in 2003.

To aid with management considerations for exploited cetaceans, Wade (1998) developed the Potential Biological Removal (PBR) method to calculate a threshold for human-related mortality. This method uses an estimate of abundance for the population subject to mortality and, for endangered species, a conservative recovery factor developed through population simulations that will allow the population to recover at a rate close to its biological maximum (\( R_{max} \)). A default \( R_{max} \) of 0.04 has been recommended for cetaceans based on available observations and estimates from life history characteristics (Wade 1998), and is the value used under the US Marine Mammal Protection Act (Wade and Angliss 1997). This default is meant to be a reasonable value for most cetaceans, however, a species-specific estimate is recommended if available. For Hector’s and Maui’s dolphins, an \( R_{max} \) of 0.018 was estimated based on empirical survival rates, the most optimistic empirical parameters for age at first reproduction and calving interval, and marine mammal survivorship curves (Slooten and Lad 1991). Based on the 2004 Maui’s dolphin abundance estimate of 111 from aerial surveys (Slooten et al. 2006), Slooten and Dawson (2008) calculated a PBR of 0.07 (one dolphin every 14.3 years) using the species-specific \( R_{max} \) of 0.018. For comparison, they also used the cetacean default \( R_{max} \) of 0.04 to calculate a PBR of 0.16 (one dolphin every 6.3 years).

Here, we estimate the abundance of Maui’s dolphins using genotype recapture records from more intensive sampling over a larger area, and update the PBR as a guide to the limit of human-related mortality for Maui’s dolphins.
3.3. Methods

Skin samples were collected from Maui’s dolphins by dart-biopsy (Krützen et al. 2002) during two periods of dedicated small-boat surveys from 4 February to 2 March 2010 and from 14 February to 10 March 2011 (Figure 3.1; see Oremus et al. 2012). The survey area along the west coast of New Zealand’s North Island extended from Bayly’s Beach to New Plymouth, with effort concentrated primarily within 2 km of shore in accordance with the austral summer distribution of Maui’s dolphins (Figure 3.1; Oremus et al. 2012). Calves, approximately one-half or less the size of an adult (assumed to be <1 year old; Webster et al. 2010), were excluded from biopsy sampling. Standard methods for DNA processing and individual identification based on DNA profiles (including 21 microsatellites) were applied to the 73 skin samples that were collected, as described by Oremus et al. (2012) and Hamner et al. (2014). Based on a low probability of identity \( P_{\text{ID}} = 8.5 \times 10^{-8} \) and probability of identity for siblings \( P_{\text{ID,sib}} = 4.4 \times 10^{-4} \), 39 Maui’s dolphin individuals were identified, 11 of which were sampled in both years (Table 3.2; Oremus et al. 2012, Hamner et al. 2014). The 73 samples also included five samples from two individual Hector’s dolphins \( (C. h. hectori) \) that were identified by mitochondrial DNA control region haplotypes and microsatellite genotype assignment (Hamner et al. 2014). These two Hector’s dolphins were excluded from the analyses presented here.

Using the Maui’s dolphin individuals sampled in 2010 and 2011, we estimated abundance with the Chapman-corrected Lincoln-Petersen estimator (Chapman 1951). This method assumes that: the population is geographically and demographically closed; all animals are equally likely to be sampled in each occasion; and genotypes are read correctly. Chao’s (1989) method for sparse data was used to calculate log-normal 95% confidence limits.

We calculated the PBR for Maui’s dolphins according to Wade (1998) using the 2010-11 abundance estimate and a recovery factor value of 0.1. Following Slooten and Dawson (2008), two values of \( R_{\text{max}} \) were used: \( R_{\text{max}} = 0.04 \), the default value recommended for
cetacean populations (Wade and Angliss 1997, Wade 1998), and \( R_{\text{max}} = 0.018 \), as estimated for Maui’s and Hector’s dolphins (Slooten and Lad 1991).

### 3.4. Results and Discussion

#### 3.4.1. Abundance

We used annual recapture histories for the 39 Maui’s dolphins to calculate an abundance of \( N = 55 \) (95% CL = 48, 69) individuals approximately age 1+. This application of genotype-recapture confirmed the extremely low abundance of the subspecies with higher precision than methods previously implemented (Table 3.1).

We consider our abundance estimate for the Maui’s dolphin to be generally robust to the assumptions of the Lincoln-Petersen model, listed above (Williams et al. 2002). Individual identification by multi-locus genotypes provides a universal permanent tag, but genotyping error has the potential to negatively bias the estimate if an error causes a genotype to match that of another individual, or to positively bias the estimate if an error creates a false new genotype. A sufficiently low \( P_{(ID)} \) and \( P_{(ID)\text{sib}} \), along with our use of controls and rigorous error checking minimize the potential for incorrect identification of individuals (see Oremus et al. 2012, Hamner et al. 2014).

The assumption of demographic and geographic population closure would be violated by (a) the loss of individuals between the 2010 and 2011 sampling occasions by death or emigration, or (b) the addition of individuals between the two occasions by recruitment (i.e., calves born in 2010 growing large enough to be sampled in 2011) or immigration. If either only losses or only additions occurred, a negligible clarification of the time of reference, to 2010 or 2011 respectively, would result. If both removals and additions occurred between the two occasions, the abundance estimate of the living population would be positively biased; however, our study was designed to minimize such bias by considering the life history characteristics and population structure of the Maui’s dolphin.
Therefore, in a strict sense our abundance estimate applies to the population of Maui’s dolphins alive and approximately age 1+ at some point during the study period.

Although the strict assumption of a demographically closed population is violated for most studies of wild populations, the short one-year interval between our two sampling occasions and the two to three year calving interval of these dolphins (Slooten and Lad 1991) minimizes the recruitment of individuals for sampling in the second occasion. The death of one male Maui’s dolphin was documented during our study period as a recovered carcass found floating off Raglan in 2010 (NZ Department of Conservation incident code: H202/10). There was no obvious cause of death and the individual was not known from previous genotyping (New Zealand Department of Conservation 2012). As this individual was found dead in the interval between the two sampling occasions, it was not available for recapture and was excluded from the abundance estimate.

The potential for violation of geographic closure was considered unlikely given the extent of the surveys, the limited remnant range of Maui’s dolphins, and the absence of local population structure (Pichler 2002, Hamner et al. 2012, Oremus et al. 2012). The genetic identification of two immigrant Hector’s dolphins among the Maui’s dolphins was unexpected (Hamner et al. 2014), and would have contributed to a positive bias in the estimate if undetected. However, these Hector’s dolphins were identified and excluded from the analyses presented here, and there was no evidence of interbreeding at the time of our study.

The assumption that all individuals are equally likely to be sampled within each occasion can be violated if individuals exhibit transience, trap (sampling) response, or other characteristics that create individual differences in their sampling probability. Transience (temporary emigration) seems unlikely for Maui’s dolphins given the reasons discussed above regarding geographic closure. A trap-shy response to sampling would positively bias the abundance estimate, however, no evidence for this was observed, as dolphins remained in the vicinity of the boat following sampling. By focusing our sampling into
approximately one month during each of two austral summer seasons, we allowed for the randomization of individuals within the Maui’s dolphin distribution between the sampling occasions. This is reflected by the movement of individuals up to 80 km and between areas with high sighting densities (Oremus et al. 2012). While a few Maui’s dolphin sightings have occurred beyond the offshore boundary of the surveys (New Zealand Minstry for Primary Industries and Department of Conservation 2012), an offshore stratification preventing the randomization of individuals between the sampling years seems unlikely. Finally, although our two-occasion sampling design does not allow for statistical tests of heterogeneity in individual sampling probabilities, no significant evidence for violation of this assumption was found by a previous genotype recapture study of Maui’s dolphins, which included less systematic sampling effort distributed over five summer sampling occasions from 2001 to 2007 (Baker et al. 2013).

Although a sampling design including more than two occasions would have been preferable in some regards, it was not desirable or necessary for this particular case. Due to the critically endangered status of the Maui’s dolphin, it was preferable to minimize our interaction by constraining the sampling to two occasions. By concentrating our dedicated survey effort into two one-month sampling occasions one year apart, we were able to achieve extensive coverage of the distribution of Maui’s dolphins and high capture probabilities within each of the occasions ($p_{2010} = 0.44; p_{2011} = 0.47$). These showed great improvement over the capture probabilities (range $p = 0.037$ to 0.243) estimated from open-population models used in the previous multi-year study (Baker et al. 2013). Furthermore, the high precision ($CV = 0.15$) achieved by our two-occasion abundance estimate meant that the additional resources and interaction with Maui’s dolphins required for additional occasions were not justified for the small increase in precision that might have resulted.

3.4.2. Potential biological removal

Using our abundance estimate and the default value of $R_{max} = 0.04$ for cetaceans (Wade 1998), we calculated the PBR for Maui’s dolphins to be 0.10, or 1 dolphin every 10
years. Using $R_{\text{max}} = 0.018$, as estimated for Hector’s dolphins (Slooten and Lad 1991), the PBR was 0.044, or 1 dolphin every 23 years. This suggests that the remnant population of Maui’s dolphins is not likely to show recovery if anthropogenic mortality causes one or more dolphin deaths every 10 to 23 years. Given that the PBR calculation is implicitly deterministic, and thus does not account for the increased threat of extinction through stochastic processes or depensation, it does not represent a threshold above which extinction is certain, or below which survival is assured (Wade 1998). A population viability analysis could provide further insight into the probability of events outside the control of management (Wade 1998).

### 3.4.3. Conservation implications

The results presented here were reported first to the New Zealand government, and contributed to accelerating the review of the Maui’s Dolphin Threat Management Plan (New Zealand Ministry for Primary Industries and Department of Conservation 2012). Additionally, the deaths of two dolphins in 2012 near Cape Egmont – south of the set net and trawling bans implemented in 2008 – confirmed the presence of Hector’s (Hamner et al. 2014) and perhaps Maui’s dolphins in this area. These deaths led to an extension of commercial and recreational set net restrictions out to 7 NM from the former boundary at Pariokariwa Point south to the Waiwhakaiho River, and extending further south to Hawera out to 2 NM from shore with observers required for commercial set netting between 2 and 7 NM (New Zealand Ministry for Primary Industries 2012, New Zealand Department of Conservation & Ministry for Primary Industries 2013). This decision will reduce entanglement risk to Maui’s dolphins utilizing the southern part of their distribution, as well as any Hector’s dolphins that disperse north into that area (see Hamner et al. 2014).

Despite its critically low abundance, we do not consider the Maui’s dolphin to be doomed to extinction. The population appears to be maintaining an equal sex ratio and connectivity across the remaining distribution by individual movements up to 80 km
(Oremus et al. 2012). Although there is currently no evidence of interbreeding between the two subspecies, the documentation of Hector’s dolphins naturally dispersing to the North Island (Hamner et al. 2014) provides the potential for enhancing the low genetic diversity of the Maui’s dolphin and preserving the species as part of the west coast North Island ecosystem.

### 3.5. Acknowledgments

This work was funded by the New Zealand Department of Conservation and completed under UniServices Contracts 17095 and 30642. Thanks to: all involved with sample collection, especially C. Duffy, K. McLeod, G. Hickman, B. Williams, S. Watts, C. Lilley, D. Patterson, K. Hillock, E. Carroll, D. Heimeier, and E. Brown; S. Lavery and the University of Auckland Molecular Ecology Lab. We also thank local iwi and Department of Conservation Area Offices of Auckland, Kauri Coast, Maniapoto, Taranaki, Waikato, and Warkworth for supporting this work. Samples were collected under permit RNW/HO/2009/03 from the Department of Conservation and University of Auckland Animal Ethics Protocol AEC/02/2008/R658 to CSB. The manuscript was improved by comments provided by four anonymous reviewers.

### 3.6. References


Figure 3.1. Maui’s dolphin sample locations and the coastline along the North Island of New Zealand covered by the 2010 and 2011 surveys (shaded in inset; Oremus et al. 2012).
Table 3.1. Maui’s dolphin abundance (N) estimates for 1985 to 2011 using a variety of methods. Associated 95% confidence limits (CL) and coefficients of variation (CV) are also included, unless not reported (nr) by the source.

<table>
<thead>
<tr>
<th>Method</th>
<th>Applicable year(s)</th>
<th>N</th>
<th>95% CL</th>
<th>CV</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boat line-transect</td>
<td>1985</td>
<td>134</td>
<td>nr</td>
<td>nr</td>
<td>Dawson and Slooten 1988</td>
</tr>
<tr>
<td>Computer modeling</td>
<td>1985</td>
<td>140</td>
<td>46 - 280</td>
<td>nr</td>
<td>Martien et al. 1999</td>
</tr>
<tr>
<td>Boat line-transect</td>
<td>1998</td>
<td>80</td>
<td>nr</td>
<td>nr</td>
<td>Russell 1999</td>
</tr>
<tr>
<td>Aerial line-transect</td>
<td>2001/02</td>
<td>75</td>
<td>48 - 130</td>
<td>0.24</td>
<td>Ferreira and Roberts 2003</td>
</tr>
<tr>
<td>Genotype recapture</td>
<td>2003</td>
<td>69</td>
<td>38 - 125</td>
<td>nr</td>
<td>Baker et al. 2013</td>
</tr>
<tr>
<td>Aerial line-transect</td>
<td>2004</td>
<td>111</td>
<td>48 - 252</td>
<td>0.44</td>
<td>Slooten et al. 2006</td>
</tr>
<tr>
<td>Genotype recapture</td>
<td>2010-11</td>
<td>55</td>
<td>48 - 69</td>
<td>0.15</td>
<td>current study</td>
</tr>
</tbody>
</table>

Table 3.2. Maui’s dolphin individuals identified from samples collected during small-boat surveys along the west coast of the New Zealand’s North Island from 4 February to 2 March 2010 and 14 February to 10 March 2011 (Oremus et al. 2012).

<table>
<thead>
<tr>
<th></th>
<th>2010</th>
<th>2011</th>
<th>Genotype recaptures</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of surveys</td>
<td>12</td>
<td>11</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>Distance travelled (km)</td>
<td>2117</td>
<td>1893</td>
<td></td>
<td>4010</td>
</tr>
<tr>
<td>Samples*</td>
<td>37</td>
<td>36</td>
<td></td>
<td>73</td>
</tr>
<tr>
<td>Maui's dolphin individuals</td>
<td>24</td>
<td>26</td>
<td>11</td>
<td>39</td>
</tr>
<tr>
<td>Females</td>
<td>14</td>
<td>15</td>
<td>6</td>
<td>23</td>
</tr>
<tr>
<td>Males</td>
<td>10</td>
<td>11</td>
<td>5</td>
<td>16</td>
</tr>
</tbody>
</table>

*Includes five samples, representing two individual Hector’s dolphins that were excluded from the current work (Hamner et al. 2014).
4. Comparing estimates of effective population size ($N_e$) and abundance from microsatellite genotyping of New Zealand endemic Maui’s and Hector’s dolphins

Rebecca M. Hamner

Intended for publication with the following co-authors:
Robin Waples, Rochelle Constantine, Rob Mattlin, and C. Scott Baker

“We shall see that, although the basic concept is elegantly simple, almost everything else involving effective population size is much more complicated.”
(Waples 2002, p. 148)
4.1. Abstract

Abundance and effective population size \((N_e)\) are two parameters of great interest for monitoring endangered species. The use of genotype mark-recapture analysis to estimate abundance provides the additional benefit of a genetic dataset that can be used to estimate the \(N_e\) of the parental generation. However, for iteroparous species with overlapping generations, where different age classes cannot be distinguished, interpretation and comparison of \(N_e\) to other taxa is still challenging. Here, we estimate \(N_e\) in comparison to the abundance of individuals age \(1^+ (N_{1^+})\) for the critically endangered Maui’s dolphin \((Cephalorhynchus hectori maui)\) subspecies and the population of endangered Hector’s dolphins \((C. h. hectori)\) in Cloudy Bay, New Zealand. Both populations exhibited a high \(N_e\) relative to \(N_{1^+}\), which is consistent with expectations given their life history characteristics and the limited data available for other dolphin species. As expected, the small remnant population of Maui’s dolphins had much lower \(N_e\) estimated from linkage disequilibrium \((N_e = 61, 95\% \text{ CL} = 29 - 338)\) and \(N_{1^+}\) \((55, 95\% \text{ CL} = 48 - 69)\) than the population of its closely related sister subspecies, the Hector’s dolphin in Cloudy Bay \((N_e = 207, 95\% \text{ CL} = 127 - 447; N_{1^+} = 272, 95\% \text{ CL} = 236 - 323)\). The Maui’s dolphin also had a slightly higher \(N_e/N_{1^+}\) ratio when compared to the Hector’s dolphin, which is consistent with a recent decline in the Maui’s dolphin. Although the point estimates of both \(N_e\) and \(N_{1^+}\) decreased between the two Maui’s dolphin datasets, the confidence intervals widely overlapped. This is not surprising given the low power to detect a low to moderate decline over the short interval (<1 generation) between the two sampling periods. Using the \(N_{1^+}\) from Maui’s 01-07 and the \(N_e\) from Maui’s 10-11 to account for the generational lag, we approximate \(N_e/N_{1^+}\) to be 0.884. When this ratio is multiplied by the Maui’s 10-11 abundance of 55, it results in an approximate contemporary \(N_e = 49\) for the Maui’s dolphin. Compared to recommendations for minimum viable effective population sizes, the Maui’s dolphin has declined below the suggested threshold of \(N_e = 50\), recently increased to \(\geq 100\), to avoid inbreeding depression in the short term.

Additionally, both the Maui’s dolphin and Cloudy Bay Hector’s dolphin populations are below the recommended threshold of \(N_e = 500\), recently increased to \(\geq 1000\), thought to be
necessary to preserve long-term evolutionary potential. This is less of a concern for the Cloudy Bay Hector’s population, which is thought to maintain gene flow with neighboring populations. However, for the small, isolated Maui’s dolphin population, inbreeding depression is likely to be an increasing concern. Furthermore, each Maui’s dolphin individual holds a disproportionate amount of the total genetic variation of the subspecies and would represent a disproportionately large demographic and genetic loss if it died before realizing its reproductive potential in the population.
4.2. Introduction

One of the most basic, and often challenging, questions to be answered for endangered species is: how many are there? This question can relate to the demographic parameter of abundance or to its genetic analogue, effective population size ($N_e$). Both provide important information for management decisions, which can become more informative when estimates for both parameters are available.

Abundance ($N$) is the traditional metric used by managers to assess the status and trend of a population, and often to evaluate the success or failure of management actions (Williams et al. 2002). The difficulty and impracticality of directly counting individuals has led to the development of a variety of approaches for estimating abundance by distance sampling or capture-mark-recapture methods (e.g., Buckland et al. 2001, Williams et al. 2002, Buckland et al. 2004, Amstrup et al. 2005). When abundance is related to effective population size, it is often restricted to the number of sexually mature adults ($N_c$), if age class can be determined (e.g., Luikart et al. 2010). The use of genotypes in place of physical tags or natural marks to identify individuals for mark-recapture analysis has the added benefit of providing genetic data that can be used to assess other parameters, including effective population size (Luikart et al. 2010).

Effective population size ($N_e$) is a central parameter in population genetics, which Wright (1938) defined as the size of an ideal population that experiences genetic change at the same rate as the population under consideration (Waples 2002). An ideal population has a constant number of diploid individuals, an equal sex ratio, random mating, non-overlapping generations, and all individuals have an equal probability of contributing an offspring to the next generation (Allendorf et al. 2013). For endangered species, effective population size is of great interest because it determines the rate at which genetic variability is lost, the rate at which inbreeding increases, and the relative evolutionary importance of selection and random genetic drift acting on a population (Waples 2002). In populations with small $N_e$, the force of selection will be dominated by genetic drift,
and greater losses of genetic diversity and increases in inbreeding per generation will occur. A multitude of approaches for estimating $N_e$ have been developed (e.g., Ewens 1982, Caballero 1994, Pudovkin et al. 1996, Waples 2006, Nomura 2008, Tallmon et al. 2008). Of these, single-sample estimators (i.e., linkage disequilibrium, Waples and Do 2008; ONeSAMP, Tallmon et al. 2008) are of most interest for the study of endangered species, as they estimate contemporary $N_e$, do not require two samples spanning a full generation, and are easily applied to data collected for genetic monitoring with multiple research objectives (Waples and Do 2010). Of particular appeal is the potential to estimate both $N_e$ and $N$ using microsatellite genotypes collected from wild populations. One caveat to using the same genetic dataset for both estimates is that the two will not be directly comparable unless a constant population size can be assumed. This is because $N_e$ will refer to the parental generation of the sample. $N_e$ might also be useful for detecting trends in endangered species, as simulations suggest that for small to moderate populations ($N < 500$), estimating $N_e$ using the linkage disequilibrium method might be more precise and accurate for detecting population trends than estimating $N$ by mark-recapture (Tallmon et al. 2010).

4.2.1. Estimating $N_e$

Although $N_e$ is of great interest for endangered species, many do not conform to the life history traits for which these estimators were designed, and therefore complicate the estimation and interpretation of results. One-sample estimators are most easily interpreted for species with short lifespans, discrete generations, and high fecundity. However, many taxa such as dolphins do not fit any of these traits; they are long-lived with overlapping generations, give birth to only a single offspring at a time, and often skip two or more years between calving (i.e., intermittent breeding). A recent analysis of 63 diverse taxa facilitated the interpretation of $N_e$ in such species by describing correlations of life history traits with the ratio of $N_e$ to $N_c$ (Waples et al. 2013). Age at maturity, adult lifespan and coefficient of variation of age-specific fecundity were found to explain most of the variation in $N_e/N_c$. The bottlenose dolphin (*Tursiops truncatus*) was among the species analyzed, and showed a high $N_e/N_c$ of 0.921. In addition to the life history correlations
identified by Waples et al. (2013), limits to litter size and intermittent breeding have also been found to influence estimates of $N_e$ (Waples and Antao 2014). However, the concurrence of intermittent breeding and constrained litter size means that different females reproduce in different breeding cycles and individual females cannot dominate a cycle, thereby reducing lifetime variance in reproductive output, and increasing $N_e$ (Waples and Antao 2014). However, only a modest increase in $N_e$ is expected, as the longevity of many animals that fit these parameters will allow ample opportunity for variance in lifetime reproductive success, even if variance is limited in the short term. Now that these correlations with life history traits have been discovered, when interpreting $N_e/N_c$ ratios as high or low, they should be compared to ratios from species with similar life history characteristics, as opposed to taxa-wide averages (e.g., Frankham 1995).

4.2.2. Maui’s and Hector’s dolphins

Hector’s dolphins (Cephalorhynchus hectori) are endemic to the coastal waters of the North and South Islands of New Zealand. Two subspecies of Hector’s dolphins are now recognized: $C. h. hectori$, which retains the common name Hector’s dolphin, and $C. h. maui$, now referred to as Maui’s dolphin (Baker et al. 2002). Hereafter, we use the term Hector’s dolphin to refer to the South Island subspecies. The Maui’s dolphin is a genetically isolated remnant population along the central west coast of the North Island (Hamner et al. 2012, Oremus et al. 2012a, Appendix III, Baker et al. 2013, Appendix II). Hector’s dolphins are distributed discontinuously around the South Island of New Zealand, with genetically differentiated regional populations along the east, west and south coasts (Hamner et al. 2012). Both subspecies experience relatively high rates of fisheries-related mortality given their low reproductive rates (Dawson 1991, Slooten and Lad 1991, Martien et al. 1999, Dawson et al. 2001, Slooten and Dawson 2008, Slooten and Davies 2012). As a consequence, the IUCN has listed the Hector’s dolphin as ‘endangered’ and the Maui’s dolphin as ‘critically endangered’ (Reeves et al. 2008).
These dolphins do not conform to the life history traits for which \( N_e \) estimators were designed. They are thought to live for a maximum of approximately 20 years and reach sexual maturity at six to nine years of age, after which females give birth to a single calf every two to three years (Slooten and Dawson 1988, Slooten and Lad 1991, Dawson and Slooten 1993). It is also not possible to distinguish age classes in order to estimate the census size of adults only. By approximately one year of age these dolphins reach adult size (Webster et al. 2010), and immature juveniles are visually indistinguishable from mature adults. Despite these challenges, the estimation of \( N_e \) and its relation to abundance is still of interest for the genetic monitoring of these subspecies.

4.2.3. Objectives

Here, we estimate the effective population size \( (N_e) \) in comparison to the abundance of individuals age \( 1^+ \) \((N_{1+})\) for the Maui’s dolphin and Hector’s dolphins in Cloudy Bay. We compare two datasets available from different time periods for the Maui’s dolphin, and as well as comparing this small remnant population with a more abundant population of its closely related sister subspecies, the Hector’s dolphin, in Cloudy Bay. This provides the rare opportunity to examine the potential effect of a bottleneck on the \( N_e/N_{1+} \) ratio.

4.3. Methods

Our work includes two datasets that use genotypes and abundance estimates from published studies of the Maui’s dolphin: Maui’s 01-07, including samples collected from 2001 to 2007 (Baker et al. 2013; Appendix II), and Maui’s 10-11, using samples collected in 2010 and 2011 (Oremus et al. 2012a, Appendix III, Hamner et al. in press, Chapter 3). The third dataset, Hector’s CB11-12, is first described here and includes samples collected from Cloudy Bay in 2011 and 2012.
4.3.1. Survey effort and sample collection

The Maui’s 01-07 dataset, included skin biopsy samples that were collected during dedicated small-boat surveys conducted during five spring/summer sampling periods between January 2001 and December 2006, as well as skin samples collected from beachcast or floating carcasses recovered by the New Zealand Department of Conservation (DOC) between January 2001 and November 2007 (Figure 4.1; additional details in Baker et al. 2013, Appendix II). The Maui’s 10-11 samples were collected during dedicated small-boat surveys in the austral summers of 2010 and 2011 (Figure 4.1; additional details in Oremus et al. 2012a, Appendix III). Additionally, one sample from a carcass found floating off Raglan in 2010 was provided by DOC (incident code: H202/10). For the Hector’s CB11-12 dataset samples were collected during dedicated small-boat surveys in the austral summers of 2011 and 2012 (Figure 4.2, Table 4.1). All skin biopsy samples were collected from free-swimming dolphins using a biopsy system with dart tips specifically designed for small dolphins (Krützen et al. 2002). Calves, approximately one-half or less the size of an adult, and assumed to be <1 year old (Webster et al. 2010), were excluded from biopsy sampling. All samples were stored in 70% ethanol at -20°C prior to tissue digestion with proteinase K followed by total cellular DNA extraction from a sub-sample using a standard phenol/chlorofom/isoamyl (PCI) protocol (Sambrook et al. 1989) as modified for small samples by Baker et al. (1994). As recommended for genetic monitoring studies (Jackson et al. 2012), the remainder of each tissue sample was archived in the New Zealand Cetacean Tissue Archive at the University of Auckland (Thompson et al. 2013).

4.3.2. DNA profiling for individual identification

DNA profiles were assembled for each sample, including genetic sex, mtDNA control region sequence, and microsatellite genotype. Sex was identified using a multiplexed PCR protocol to amplify fragments of the sry and ZFX/ZFY genes according to Gilson et al. (1998). A fragment of approximately 700 bp from the 5’ end of the maternally inherited mtDNA control region was amplified and sequenced as described by Hamner et
Geneious Pro 5.5.2 (Biomatters, Ltd.) was used to assign mtDNA haplotypes based on alignment with 576 bp reference sequences for the 28 haplotypes previously described for the species (Pichler et al. 1998, Pichler and Baker 2000, Pichler 2002, Hamner 2008, Hamner et al. 2012).

Previously published cetacean primers were used to amplify up to 21 microsatellite loci for all Maui’s dolphin samples (Hamner et al. 2014), extending the genotypes for the Maui’s 01-07 samples used by Baker et al. (2013), and up to 17 loci for the Hector’s CB11-12 samples (Table 4.2). Each locus was amplified individually according to the conditions in Table 4.2, and co-loaded with up to 5 other loci amplified from the same individual for sizing by an ABI 3130 Genetic Analyzer (Applied Biosystems). Geneious Pro v5.5.2 (BioMatters) was used to bin and visually verify the resulting size peaks. Each amplification and sizing run included a negative control to detect contamination and 7 to 10 internal control samples to standardize allele binning with previous genotyping runs. Additionally, a subset of samples were randomly selected for replicate genotyping to estimate genotyping error by dividing the number of incongruent allele calls by the total number of alleles repeated, as recommended by Bonin et al. (2004). GenAlEx v6.4 (Peakall and Smouse 2006) was used to calculate the probability of identity ($P_{ID}$) and probability of identity for siblings ($P_{ID,sib}$) for each locus as well as for all loci combined.

Microsatellite genotypes were compared for the purposes of individual identification, both within and across sampling years, using the program CERVUS 3.0.3 (Kalinowski et al. 2007). Initial comparisons allowed for mismatching of up to 5 loci (‘relaxed matching’) to prevent false exclusion due to genotyping error. Relaxed matches were examined for potential allelic dropout, as well as matching sex and mtDNA haplotype, and re-genotyped up to 3 times to confirm or correct the genotype as necessary. After review and correction, samples with identical genotypes or apparent allelic dropout at one locus, were accepted as resamples of the same individual (i.e., genotype captures and recaptures), based on a low $P_{ID}$ and $P_{ID,sib}$. Micro-Checker v.2.2.3 (Van Oosterhout et al. 2004) was used to assess the presence of null alleles. Observed and expected
heterozygosity, inbreeding coefficient ($F_{IS}$), and a test for deviations from Hardy-Weinberg equilibrium were calculated in GenAlEx v6.4 (Peakall and Smouse 2006).

4.3.3. Geographic population closure

Estimates of both $N$ and $N_e$ can be biased if a population is open to immigration or emigration. To minimize the potential for this bias, we looked for possible evidence that could refute the assumption of geographic closure. The potential for the violation of geographic closure for the Maui’s dolphin datasets was considered unlikely given the extent of the surveys, limited remnant range of the subspecies, and the absence of local population structure (Pichler 2002, Hamner et al. 2012, Oremus et al. 2012a). The sampling for Maui’s dolphins in 2010-11, however, was found to include two female Hector’s dolphin migrants (Hamner et al. 2014). These were excluded from the analyses presented here to preserve the closure of the population, as there was no evidence of interbreeding at the time of our study.

Cloudy Bay is thought to have some level of gene flow with adjacent populations of Hector’s dolphins (Hamner et al. 2012), so we investigated the potential for significant change in the population of dolphins using our study area between our two sampling years. We assessed the genetic differentiation ($F_{ST}$) between the samples collected in the two years based on both mtDNA and microsatellite data using the program Arlequin v3.5.1.2 (Excoffier and Lischer 2010). Additionally, to assess the potential presence of migrants from other regional populations in our sample, we evaluated the likely population of origin for each of the Cloudy Bay individuals using the Bayesian assignment method of Structure v2.3.2 (Pritchard et al. 2000). Hector’s and Maui’s dolphin samples collected between 1988 and 2011, and archived at the New Zealand Cetacean Tissue Archive curated at the University of Auckland (Thompson et al. 2013) were utilized as a reference dataset for population assignment to detect potential migrants from other regional populations. This included 53 Maui’s dolphin individuals identified from samples collected from 2001 to 2007 (Oremus et al. 2012a, Baker et al. 2013), and 180 Hector’s dolphin samples collected around the South Island between 1988 and 2007.
(Hamner et al. 2012). The ‘‘Use PopInfo’’ option (G = 0) was applied to run $10^6$ Markov chain Monte Carlo (MCMC) replicates following a burn-in of $10^5$ for $K = 4$.

### 4.3.4. Abundance ($N_{1+}$)

Abundance ($N_{1+}$), as estimated here, refers to the number of individuals age $1^+$. This is because calves, which are excluded from biopsy sampling, reach adult body size by the time they are approximately 1 year old (Webster et al. 2010). After this point there is no way to distinguish non-reproductive juveniles from reproductively mature adults (age 6 to 9$^+$; Slooten 1991).

The Maui’s 01-07 abundance was estimated by Baker et al. (2013) using a bespoke open-population Pradel-like model. Abundances for Maui’s 10-11 and Hector’s CB12-11 were estimated using the two-occasion Lincoln-Petersen estimator with Chapman correction (Chapman 1951), and 95% confidence limits (CL) were calculated according to Chao’s (1989) method for sparse data.

### 4.3.5. Effective population size ($N_e$)

Two methods for estimating the effective population size ($N_e$) were used: the linkage disequilibrium method (Waples and Do 2008), as implemented in NeEstimator v2 (Do et al. 2014), and the approximate Bayesian coalescence method that includes eight summary statistics related to $N_e$, including linkage disequilibrium, as implemented in ONeSAMP (Tallmon et al. 2008). Loci showing significant evidence of null alleles or deviation from Hardy-Weinberg equilibrium can bias the analyses and were excluded. For the linkage disequilibrium method, the critical value was set to exclude alleles with frequencies less than 0.02, as recommended by Waples and Do (2010). For ONeSAMP, the $N_e$ priors were set to 2 and 100 for the Maui’s dolphin dataset and 2 and 500 for the Hector’s dolphin dataset. A range of priors were also run for both datasets to assess consistency in the estimate produced (Table 4.3).
4.3.6. $N_e/N_{1+}$

$N_e/N_{1+}$ ratios were calculated for each of the datasets for comparison to each other and other published datasets. Ideally for the calculation of $N_e/N_{1+}$, a generation (12.5 years; Taylor et al. 2007) would intervene between the datasets used to estimate the two parameters, as $N_e$ estimated here refers to the effective number of parents that produced the sample. Just over half a generation passed between the collection of samples for Maui’s 01-07 (the majority of which were ≤ 2003) and Maui’s 10-11. By using the $N_{1+}$ from Maui’s 01-07 and the $N_e$ from Maui’s 10-11, we approximate $N_e/N_{1+}$ of Maui’s dolphins for the 2001-07 time period. The resulting ratio can then be multiplied by the Maui’s 10-11 abundance of 55 to approximate the contemporary $N_e$ for the Maui’s dolphin population in 2010-11.

A rough approximation of the $N_e/N_{1+}$ ratio can also be made if it can be assumed that no change in population size occurred between the generation sampled and the parental generation. This assumption is not unreasonable for Cloudy Bay, so we compared the two numbers directly to approximate the $N_e/N_{1+}$ ratio. For comparison to Hector’s CB 11-12, ratios using $N_e$ and $N_{1+}$ estimates from the same dataset were also calculated for the Maui’s dolphin datasets.

4.4. Results

4.4.1. Sample collection

Maui’s 01-07 included 70 biopsy samples collected along the west coast of the North Island during the 2001-2006 surveys, and 12 collected from recovered carcasses between 2001 and 2007 (Baker et al. 2013). Maui’s 10-11 included 68 biopsy samples collected along the west coast of the North Island during the 2010 and 2011 surveys (Oremus et al. 2012a). During these surveys, an additional five samples representing two female Hector’s dolphin migrants were also collected and are described by Hamner et al. (2014). These Hector’s dolphin migrants were excluded from the analyses presented here. Hector’s CB11-12 included 263 biopsy samples collected in Cloudy Bay during the 2011 and 2012 surveys (Table 4.1).
4.4.2. DNA profiling and individual identification

All Maui’s dolphins sampled during both time periods had the subspecies’ characteristic mtDNA haplotype ‘G’ (Oremus et al. 2012a, Appendix III, Baker et al. 2013, Appendix II). For Hector’s CB11-12, 17 mtDNA haplotypes were resolved based on 576 bp, of which 12 were previously identified in Hector’s dolphins (Pichler et al. 1998, Pichler and Baker 2000, Pichler 2002, Hamner 2008, Hamner et al. 2012); 2 were resolved from one previously identified haplotype based on the longer sequence (i.e., Cb1 and Cb2 from Cb); and 3 were newly identified (X, Y, AB; Table 4.4).

For Maui’s 01-07, the identification of 54 Maui’s dolphin individuals based on 14 loci by Baker et al. (2013, Appendix II) was confirmed by analysis of the 21-locus genotypes used here ($P_{ID} = 4.3 \times 10^{-7}$; $P_{IDsib} = 8.1 \times 10^{-4}$). One individual (CheNI058) with a low quantity of DNA had sufficient loci to be excluded as a match to other genotypes, however insufficient loci for the analysis to estimate $N_e$, leaving this dataset with 53 individuals. For Maui’s 10-11, the identification of 39 Maui’s dolphin individuals based on 21-locus genotypes ($P_{ID} = 4.0 \times 10^{-7}$; $P_{IDsib} = 8.1 \times 10^{-4}$) reported by Oremus et al. (2012a, Appendix III) and Hamner et al. (2014, Chapter 2) were reviewed and confirmed.

For Hector’s CB11-12, samples were genotyped for up to 17 microsatellite loci, with an average of 16.3 loci per sample (Table 4.2). Four of the 263 samples were atypically small skin biopsies that yielded very low quantities of DNA and only amplified for ≤3 loci. These four samples were therefore excluded from further analyses. Based on the repeated genotyping of 28 control samples (1840 alleles), the genotyping error rate was 0.1%, all of which was due to apparent allelic dropout. Considering all loci, the probability of identity ($P_{ID}$) was $6.9 \times 10^{-11}$ and probability of identity for siblings ($P_{IDsib}$) was $5.1 \times 10^{-5}$ (Table 4.2). Given this low probability of a match by chance, we assumed that unique genotypes represented individual dolphins and that samples with matching genotypes were in fact replicate samples (i.e., genotype recaptures) of the same individual. Sex and mtDNA haplotypes were subsequently compared and agreed for all of the genotype matches. From the 259 samples with informative microsatellite genotypes, a total of 148 individuals were identified.
4.4.3. Cloudy Bay population closure

No genetic differentiation was found between the Hector’s dolphin samples collected in Cloudy Bay in 2011 and 2012 (mtDNA $F_{ST} = 0, P = 0.784$; microsatellite $F_{ST} = 0, P = 0.847$). This suggests that the same genetic population was present in the area during both sampling occasions. Furthermore, population assignment did not find evidence of migrants from other regional populations (i.e., West Coast or South Coast). The Structure analysis assigned all individuals sampled in Cloudy Bay to the East Coast South Island population with high membership coefficients ($q \geq 0.9$), with the exception of one sample (Che11CB107; $q = 0.722$; Figure 4.3). However, Che11CB107 did not meet the threshold of $q = 0.6$ used by Hamner et al. (2012) to identify migrants or offspring of migrants, and so was not excluded from the dataset.

4.4.4. Genotype capture-recapture abundance

Based on the genotype recapture histories for the individuals in Maui’s 01-07, Baker et al. (2013) used a bespoke open-population Pradel-like model to estimate an abundance of $N_{I+} = 69$ (95% CL = 38 - 125) for the mid-point of the study, 2003. Based on the genotype recapture histories for Maui’s 10-11 individuals sampled alive, Hamner et al. (in press) estimated the abundance to be $N_{I+} = 55$ (95% CL = 48 - 69). Based on the genotype recapture histories for Hector’s CB11-12 individuals, we estimated the abundance to be $N_{I+} = 272$ (95% CL = 236 - 323; Table 4.5).

4.4.5. Effective population size and $N_e/N_{I+}$

Although loci with significant deviations from Hardy-Weinberg equilibrium or evidence of null alleles are not likely to affect the identification of individuals or estimation of $N_{I+}$, they could bias the estimate of $N_e$. Therefore, loci with significant evidence of these characteristics were excluded from this set of analyses, leaving 19 loci for Maui’s 01-07, 19 loci for Maui’s 10-11, and 13 loci for Cloudy Bay Hector’s 11-12 (Table 4.2). All three datasets produced relatively high estimates of $N_e$ when the linkage disequilibrium estimator was used: Maui’s 01-07 $N_e = 74$ (95% CL = 37 – 318); Maui’s 10-11 $N_e = 61$
When the ONeSAMP method was used, however, the $N_e$ estimates were lower with much narrower confidence intervals (Table 4.5). The Maui’s dolphin datasets produced relatively consistent results independent of the $N_e$ priors used, however the very narrow confidence intervals did not always overlap (Table 4.5). Using the a priori selected priors of 2-100, Maui’s 01-07 $N_e = 20$ (95% CL = 17 – 25) and Maui’s 10-11 $N_e = 13$ (95% CL = 11 – 16). Hector’s CB11-12, however, was sensitive to the priors selected, producing estimates ranging from 39 to 214, with non-overlapping confidence intervals between many of the estimates (Table 4.3c).

Using the $N_{1+}$ from Maui’s 01-07 and the $N_e$ from Maui’s 10-11 to account for the generational lag, we approximate $N_e/N_{1+}$ to be 0.884. When this ratio is multiplied by the Maui’s 10-11 abundance of 55, it results in an approximate contemporary $N_e = 49$ for the Maui’s dolphin. Assuming no change in population size for Cloudy Bay Hector’s dolphins, an approximate $N_e/N_{1+}$ ratio of 0.761 results from comparing $N_e$ and $N_{1+}$ from the same dataset. For comparison to the Hector’s CB 11-12 ratio, ratios using $N_e$ and $N_{1+}$ estimates from the same dataset were also calculated for the Maui’s dolphin datasets (Table 4.5). The $N_e/N_{1+}$ ratios using the linkage disequilibrium $N_e$ were higher for both Maui’s dolphin datasets than Hector’s CB11-12 (Table 4.5).

4.5. Discussion

The robust estimation of effective population size per generation ($N_e$) and abundance of adults ($N_c$) are not feasible for some species, and the uncertainties associated with each estimate will contribute to the uncertainty associated with their resulting ratio. However, careful interpretation and the use of related parameters, such as age 1+ abundance ($N_{1+}$), can still provide valuable insights. Although not directly comparable to published estimates of $N_e/N_c$, the $N_e/N_{1+}$ estimates produced for Maui’s and Hector’s dolphins are still useful for qualitative comparisons to the few other dolphin populations with published data and for genetic monitoring of the two subspecies.
4.5.1. Comparisons to other dolphins

An approach similar to ours for estimating $N_e$ was applied to rough-toothed dolphins ($Steno bredanensis$) off Moorea. $N_e$ was estimated by both linkage disequilibrium and ONeSAMP, and can be compared with an abundance estimate derived simultaneously by photo-ID recapture $N = 211$ (95% CL = 137 – 389; CV = 0.29; Oremus et al. 2012b). This population also showed a high $N_e/N$ ratio (0.882) using the linkage disequilibrium $N_e$, as well as a similar discrepancy between $N_e$ estimated by the linkage disequilibrium method, $N_e = 186$ (95% CL = 108 - 552), and ONeSAMP, $N_e = 95$ (95% CL = 71- 181). This pattern of discrepancy is not uncommon, but also is not universal in the literature (e.g., Beebee 2009, Saarinen et al. 2010, Van Doninck et al. 2010, Belmar-Lucero et al. 2012, Remon et al. 2012, Schregel et al. 2012, Dutta et al. 2013, Holleley et al. 2014). ONeSAMP incorporates eight summary statistics in its estimation of $N_e$, including linkage disequilibrium (Tallmon et al. 2008). This gives it the potential to be more accurate than the method based solely on linkage disequilibrium because it includes more empirical data about the population in its calculation. However, it also means there are more parameters that could potentially bias the estimate. For example, ONeSAMP assumes a stepwise mutation model, which could lead to bias if the loci analyzed do not strictly follow this model. Perhaps if increasing studies report both estimates, a comparative analysis will be able to identify correlations to the likely cause of the disagreement. As we were not able to get a consistent estimate for Hector’s CB11-12 using ONeSAMP, the rest of our discussion will focus on the linkage disequilibrium estimates.

The bottlenose dolphin ($Tursiops truncatus$) example presented by Waples et al. (2013) reports a high $N_e/N_c$ ratio of 0.921. This is similar to our cross-dataset ratio for the Maui’s dolphin (0.884) and the same-dataset ratio for rough-toothed dolphins (0.882; Oremus et al. 2012b), however, these ratios are not directly comparable, as the bottlenose dolphin ratio uses adult census size, and the other two will include some juveniles in the abundance estimate. The $N_e/N_c$ ratio for Hector’s and Maui’s dolphins will be greater than the $N_e/N_{1+}$ ratio reported here because the number of adults ($N_c$) must be lower than the number of adults plus juveniles over age 1 ($N_{1+}$). Therefore, the true $N_e/N_c$ ratios for
the Maui’s and Hector’s dolphins will be greater than the one reported for the bottlenose dolphin, perhaps exceeding 1.

Data from two populations of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) in Morton Bay, Australia, are more directly comparable to our results, in that *N* is not restricted to adults, however, they showed much lower *N*/*N* ratios (Ansmann et al. 2013). For the northern population, linkage disequilibrium *N* e = 126 (95% CL = 71 – 390) and photo-ID *N* = 446 (95% CL = 336 – 556), and for the southern population, linkage disequilibrium *N* e = 56 (95% CL = 36 – 102) and photo-ID *N* = 193 (95% CL = 181 – 207). Using these numbers, same-dataset *N*/*N* ratios would be 0.283 and 0.290, respectively, much lower than those observed for Maui’s and Hector’s dolphins.

It is possible that some populations of bottlenose dolphins generally have lower *N*/*N* ratios than Hector’s and Maui’s because they exhibit behaviors such as intraspecific competition, male alliances, and infanticide (e.g., Connor et al. 1996, Dunn et al. 2002, Krützen et al. 2004), which may result in higher reproductive variance, particularly for males. There are no reported observations of these behaviors in Maui’s and Hector’s dolphins, which could potentially have very low reproductive variance because of reduced abundances, lower competition for resources and therefore increased survival of offspring (e.g., Saarinen et al. 2010).

### 4.5.2. Maui’s vs Hector’s comparisons

Both *N* e and *N* 1+ are much lower for the remnant population of Maui’s dolphins than the population of Hector’s dolphins in Cloudy Bay. While we expect this pattern, there is potential for the Cloudy Bay Hector’s *N* e estimate to be biased high by gene flow with adjacent populations. If migration rates of ~10% or higher occur, this can result in an *N* e estimate representing the metapopulation *N* e (Waples and Do 2010). We did not identify any migrants from outside the East Coast population in our sample, but our analysis lacks precision to identify migrants from other local populations within the East Coast, such as Clifford Bay or Kaikoura. While step-wise connectivity with neighboring populations
has been previously suggested (Hamner et al. 2012), it is not known if it is facilitated by one-off permanent dispersal or more temporary seasonal shifts in home-range, or perhaps both. In either case, such connectivity is promising for maintaining the genetic diversity and evolutionary potential of the population of Hector’s dolphins in Cloudy Bay.

The $N_e/N_{1+}$ ratios are also higher for both Maui’s dolphin datasets than for the Hector’s dolphin. Linkage disequilibrium can require several generations to decay (Waples 2005, Waples 2006), therefore a large $N_e/N_{1+}$ could be reflecting information on $N_e$ of up to a few generations in the past, but $N_{1+}$ of the present. A larger $N_e$ relative to $N_{1+}$ is consistent with a recent decline (Crandall et al. 1999), and is the likely reason why the $N_e/N_{1+}$ ratios for the Maui’s dolphin are slightly higher than that for the Cloudy Bay Hector’s dolphin.

4.5.3. Maui’s dolphin monitoring
The point estimates for both $N_e$ and $N_{1+}$ were consistent in suggesting a decline over time, however, both had confidence limits that largely overlap between the two time periods (see also trend analysis in Appendix VI). Given the short time interval (<1 generation) between the two datasets, power to detect a significant trend is likely quite low. In general, for a modest trend to be detectable in $N_e$, an interval of at least five generations is required (Tallmon et al. 2010). This translates to approximately 60 years for Maui’s dolphins, assuming a generation time of 12.5 years (Taylor et al. 2007). The long generation time and overlap of generations is likely to be buffering the small population of Maui’s dolphins from a more severe loss of genetic diversity. Similar patterns have been reported for a variety of endangered species reduced to small numbers, including the greater one-horned rhinoceros (Dinerstein and McCracken 1990), white-tailed eagle (Hailer et al. 2006) and copper redhorse (a fish; Lippe et al. 2006).

4.5.4. Conservation implications
Compared to recommendations for minimum viable effective population sizes (Franklin 1980; Frankham et al. 2014), the approximated contemporary $N_e = 49$ for the Maui’s
dolphin is below the suggested threshold ($N_e = 50$, recently increased to $\geq 100$) to avoid inbreeding depression in the short term (5 generations $\sim 62.5$ years for Maui’s and Hector’s dolphins). Additionally, the $N_e$ estimates and their 95% confidence limits for both the Maui’s dolphin and Cloudy Bay Hector’s dolphin populations are below the recommended threshold ($N_e = 500$, recently increased to $\geq 1000$) thought to be necessary to preserve long-term evolutionary potential in perpetuity. This is most concerning for the isolated population of Maui’s dolphins, as the Cloudy Bay Hector’s dolphins are thought to have some gene flow with neighboring populations (Hamner et al. 2012). Such gene flow would help to facilitate matings between unrelated individuals and maintain genetic diversity in the population. With no gene flow, the genetic diversity of the small remnant Maui’s dolphin population will continue to be lost at rate of $1/2N_e$ per generation, and the negative effects of inbreeding depression will likely increase. If the recently detected Hector’s dolphin immigrants (Hamner et al. 2014) are found to interbreed with the Maui’s dolphins, the negative effects on the Maui’s dolphin population could be ameliorated, at least temporarily. The high $N_e/N_{T+}$ ratio observed currently for the Maui’s dolphin is somewhat promising for the survival of the subspecies. However, it also means that each individual holds a disproportionate amount of the total genetic variation left in the subspecies and would represent a disproportionately large demographic and genetic loss if it died before realizing its reproductive potential in the population.

4.6. Acknowledgments

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4.7. References


Table 4.1. Survey effort and biopsy samples collected from Hector’s dolphins in Cloudy Bay, New Zealand.

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<th></th>
<th>2011</th>
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<td>18 - 24 Feb</td>
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Table 4.2. Microsatellite loci used for the individual identification, estimation of abundance and effective population size for (a) Maui’s dolphins sampled between 2001 and 2007, (b) Maui’s dolphins sampled in 2010 and 2011, and (c) Hector’s dolphins in Cloudy Bay sampled in 2011 and 2012.

(a) Maui’s 01-07

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Overall  82  mean=2.4  4.3E-07  8.1E-04  53  mean=0.33  mean=0.32  mean=-0.035
### (b) Maui’s 10-11

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Table 4.3. Estimates of the effective population size ($N_e$) using multiple priors in the program ONeSAMP for (a) Maui’s dolphins sampled between 2001-2007, (b) Maui’s dolphins sampled in 2010-2011, and (c) Cloudy Bay Hector’s dolphins sampled in 2011-2012. The values in bold were reported in the text.

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(b) Maui’s 10-11

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<td>179.5</td>
</tr>
<tr>
<td>20</td>
<td>350</td>
<td>121.7</td>
<td>101.5</td>
<td>207.2</td>
</tr>
<tr>
<td>20</td>
<td>350</td>
<td>105.7</td>
<td>88.8</td>
<td>173.7</td>
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<td>20</td>
<td>500</td>
<td>214.4</td>
<td>167.9</td>
<td>396.0</td>
</tr>
<tr>
<td>30</td>
<td>250</td>
<td>134.4</td>
<td>116.2</td>
<td>189.4</td>
</tr>
<tr>
<td>30</td>
<td>250</td>
<td>151.7</td>
<td>130.9</td>
<td>206.2</td>
</tr>
</tbody>
</table>
Table 4.4. Mitochondrial DNA control region (576 bp) haplotypes for Hector's dolphin individuals sampled in Cloudy Bay in 2011 and 2012. The overall total for both years after removing replicate individuals is also shown.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>2011</th>
<th>2012</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Ca</td>
<td>28</td>
<td>23</td>
<td>48</td>
</tr>
<tr>
<td>Cb1</td>
<td>9</td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>Cb2</td>
<td>4</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Ha</td>
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<td>1</td>
</tr>
<tr>
<td>Hb</td>
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<tr>
<td>Ia</td>
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<td>Ib</td>
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<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Ja</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Jb</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>P</td>
<td>3</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>W</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>X</td>
<td>4</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Y</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>AB</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>86</strong></td>
<td><strong>94</strong></td>
<td><strong>152</strong></td>
</tr>
</tbody>
</table>
Table 4.5. Abundance of individuals age 1+ ($N_{1+}$) and effective population size ($N_e$) for Maui’s dolphins sampled between 2001 and 2007 (Maui’s 01-07), Maui’s dolphins sampled in 2010 and 2011 (Maui’s 10-11), and Hector’s dolphins sampled in Cloudy Bay in 2011 and 2012 (Hector’s CB11-12). The number of independent comparisons (Indep. comp.) and correlation coefficients ($r^2$) are shown for the linkage disequilibrium analysis.

<table>
<thead>
<tr>
<th></th>
<th>Genotype recapture</th>
<th>Linkage Disequilibrium</th>
<th>ONeSAMP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>$N_{1+}$</td>
<td>95% CL</td>
</tr>
<tr>
<td>Maui’s 01-07</td>
<td>53</td>
<td>69(^a)</td>
<td>38 - 125</td>
</tr>
<tr>
<td>Maui’s 10-11</td>
<td>40</td>
<td>55(^b)</td>
<td>48 - 69</td>
</tr>
<tr>
<td>Hector’s CB11-12</td>
<td>148</td>
<td>272</td>
<td>236 - 323</td>
</tr>
</tbody>
</table>

\(^a\)Baker et al. 2013, Appendix II
\(^b\)Hamner et al. in press, Chapter 3
Figure 4.1. Survey area (shaded orange; distance from shore not to scale) and locations of biopsy samples collected from Maui’s dolphins along the west coast of the North Island, New Zealand, in the austral summers of 2010 and 2011 (Oremus et al. 2012a, Appendix III). The general areas from which samples were collected from 2001 to 2007 (Baker et al. 2013, Appendix II) are also indicated as “n = #” between the dashed lines demarking the areas in the lower left inset.
Figure 4.2. Survey area (shaded red) and locations of biopsy samples collected from Hector’s dolphins in Cloudy Bay, New Zealand, in the austral summers of 2011 and 2012.
Figure 4.3. Assignment of Hector’s dolphin individuals sampled in Cloudy Bay ($n = 148$, between gray arrows) to the three regional populations of Hector’s dolphin or the Maui’s dolphin using the program Structure ($k = 4$) and a reference dataset of Hector’s and Maui’s dolphin genotypes (Hamner et al. 2012).
5. **A deep spot in a shallow gene pool: the genetic characteristics of the remnant population of Maui’s dolphins, as compared with Hector’s dolphins in Cloudy Bay**

Rebecca M. Hamner

Intended for publication with C. Scott Baker as a co-author

5.1. **Abstract**

The very low abundance and effective population size of the Maui’s dolphin (*Cephalorhynchus hectori maui*) are at levels where genetic factors could begin to increase the subspecies’ risk of extinction. Here we assess a range of genetic characteristics of the critically endangered remnant population of Maui’s dolphins in comparison with the more abundant, but still endangered, population of its sister subspecies, the Hector’s dolphin in Cloudy Bay. The current population of the critically endangered Maui’s dolphin has significantly fewer alleles (average 4 alleles/locus) and lower heterozygosity ($H_o = 0.316$, $H_e = 0.319$) than the endangered population of its sister subspecies, the Hector’s dolphin in Cloudy Bay (average 7 alleles/locus, $H_o = 0.500$, $H_e = 0.495$; all $P < 0.001$). The observed and expected heterozygosity, internal relatedness, and $F_{IS}$ of Maui’s dolphins showed patterns consistent with a decline of the subspecies, although none differed significantly over the short time interval between the two datasets collected in 2001-07 and 2010-11. If the Maui’s dolphin remains at low numbers and isolated, the already low genetic diversity and heterozygosity will likely continue to decline, further reducing the evolutionary potential of the subspecies and increasing its risk of extinction. Interbreeding with the recently identified Hector’s dolphin immigrants could result in genetic restoration. Interestingly, one microsatellite locus (PPHO104) had anomalously high diversity and appears to be influenced by strong diversifying selection in Hector’s and Maui’s dolphins. This locus could potentially offer clues to an inbreeding avoidance mechanism, but additional investigation is required. Continued genetic monitoring and examination of recovered carcasses for phenotypic signs of inbreeding are important for gauging genetic threats to the persistence of Maui’s dolphins.
5.2. Introduction

The Maui’s dolphin (*Cephalorhynchus hectori maui*) is one of the most endangered cetaceans in the world. It is endemic to the North Island of New Zealand, where its limited distribution has contracted to approximately 300 km along the central part of the west coast, with most sightings occurring within 150 km (Oremus *et al.* 2012). It has reached a critically low abundance estimated to be 55 individuals age 1+ (95% CL: 48-69; Hamner *et al.* in press, Chapter 3), with a similarly low effective population size estimated to be $N_e = 61$ (95% CL: 29 – 338) for the parental generation and approximated to be $N_e = 49$ for the current population (Chapter 4). This puts the current Maui’s dolphin population below the recommended threshold $N_e$ of 500, recently increased to $N_e \geq 1000$, thought to be necessary to preserve long-term evolutionary potential, as well as below the threshold $N_e$ of 50, recently increased to $N_e \geq 100$, thought to be necessary to avoid inbreeding depression (Franklin 1980; Frankham *et al.* 2014). In such small populations genetic factors begin to contribute to extinction risk (Frankham 2005).

A decline in the abundance of a population, or demographic bottleneck, is often accompanied by a genetic bottleneck, or decline in allelic diversity and heterozygosity (Nei *et al.* 1975). Alleles are easily lost during a bottleneck, particularly from highly diverse loci. Heterozygosity is relatively less sensitive to bottlenecks, but losses will affect the ability of a population to evolve in the short-term (Allendorf *et al.* 2013). The magnitude and duration of a bottleneck, along with life history characteristics of the species of interest, will determine the magnitude of the impact and our ability to significantly detect a bottleneck (Nei *et al.* 1975, Peery *et al.* 2012). The greatest losses will occur for populations that are reduced by proportionally larger amounts, held at lower numbers for longer durations, and have low potential growth rates.

When the size and genetic diversity of a population decline to low levels, extinction risk will increase, in part, due to an increase in inbreeding (Frankham 2005). The term inbreeding can be defined in several contexts, but in general, it describes an increase in
homozygosity (a.k.a. decrease in heterozygosity) that results from matings between related individuals (Keller and Waller 2002). The inbreeding coefficient, or probability that two alleles at a locus within an individual will be identical by descent, will increase more quickly in smaller populations, at a rate of \(1/2N_e\) per generation. In small populations, ancestral alleles and new mutations are eventually either eliminated by natural selection or drift to fixation by random chance. However, as a population declines the efficiency of selection is reduced, which increases the rate of fixation and accumulation of deleterious mutations that could otherwise be eliminated by selection. Therefore, in small isolated populations, even if individuals were able to avoid consanguineous matings, genetic drift will still cause the loss of alleles, fixation of mildly deleterious mutations, and an increase in homozygosity from generation to generation. As homozygosity increases, the expression of accumulated mildly deleterious mutations increases, which can cause a reduction in fitness, referred to as inbreeding depression (Keller and Waller 2002). In extreme situations, an extinction vortex or mutational meltdown, can be created, whereby the effects of inbreeding depression cause a further reduction in population size, which leads to even more inbreeding and accumulation of deleterious alleles, and so on until extinction (Gilpin and Soulé 1986, Lynch et al. 1995). For small populations of low fecundity species (e.g., mammals, birds), the expected cumulative build-up of deleterious mutations appears sufficient to cause extinction by mutational meltdown within 10s to 100s of generations (Lynch et al. 1995).

A well-documented example of inbreeding depression occurring in a wild population is the Florida panther, which was reduced to approximately 30 individuals in the 1990s (Pimm et al. 2006). Inbreeding led to reduced reproductive success resulting from the prevalence of undescended testicles, as well as increased expression of more benign recessive traits for tail kinks and cowlicks (Hedrick 1995). However, as demonstrated by the Florida panther, the introduction of unrelated individuals with novel genetic diversity from another conspecific population can avert the extinction vortex and lead to genetic rescue (Pimm et al. 2006). Genetic rescue can also occur from the natural re-establishment of gene flow among populations. A small, inbred wolf population in
Scandinavia demonstrated that even just one immigrant can cause significant increases in allelic diversity, heterozygosity, inbreeding avoidance, and subsequent exponential population growth (Vila et al. 2003).

Bottlenecks that reduce abundance gradually over a long period of time can allow selection to purge deleterious alleles while the population is still large enough, giving it time to adapt to having a low abundance. In such cases, the serious impacts of inbreeding depression and extinction vortex may be avoided, allowing populations to persist at low abundances with low genetic diversity for long periods of time without evidence of inbreeding depression (Lande 1988). For example, the small population of tuatara on North Brother Island has persisted in isolation on this 4 hectare island for approximately 10,000 years (~200 generations) (Allendorf et al. 2013). This population has much lower genetic diversity than populations on other islands, which is thought to result from a historical founder effect followed by a demographic bottleneck in the 1870s (MacAvoy et al. 2006). Although severe inbreeding depression is not evident in this population, a decline in body condition over the last 45 years suggests that inbreeding is beginning to affect the population.

Although genetic drift will outweigh selection in determining the alleles maintained in a small population, signatures of past selection may still be detectable for generations and very strong selective forces (i.e., strong correlation with mortality) might continue to act. This seems to be the case for the San Nicolas Island fox, which was monomorphic for all neutral loci examined, but still maintained diversity at immunologically important major histocompatibility complex loci (Aguilar et al. 2004).

Genetic patterns in small populations are highly stochastic, making it difficult to predict their relative role in contributing to extinction (Frankham 2005). Therefore, assessing and monitoring genetic factors in small populations of conservation concern is important for informing and evaluating adaptive management decisions. Here we assess a range of genetic characteristics of the critically endangered remnant population of Maui’s dolphins.
in comparison with a more abundant, but still endangered, population of its sister subspecies, the Hector’s dolphin in Cloudy Bay. Although still in the early stages of genetic monitoring, we also assess preliminary patterns across a short time interval between two Maui’s dolphin datasets collected in 2001-07 and 2010-11. Finally, we describe the anomalously high level of diversity at one microsatellite locus that appears to be influenced by selection.

5.3. Methods

Three previously described genetic datasets, including up to 26 microsatellite loci, were used for this work. The Maui’s 01-07 dataset includes samples collected along the west coast of the North Island of New Zealand between 2001 and 2007, for which sampling, genotyping and identification of 54 individuals were described by Baker et al. (2013; Appendix II) and genotypes were extended in Chapter 4 of this dissertation. One of the 54 individuals (CheNI058) with a low quantity of DNA, had sufficient loci to be excluded as a match to other genotypes, however not enough to be included here, leaving this dataset with 53 individuals. The Maui’s 10-11 dataset includes samples collected along the west coast of the North Island in the austral summers of 2010 and 2011, for which sampling, genotyping and identification of 39 Maui’s dolphin individuals were described by Oremus et al. (2012; Appendix III) and genotypes were extended by Hamner et al. (2014; Chapter 2). This dataset also includes one individual recovered as a floating carcass (NZ Department of Conservation incident code: H202/10). Seven Maui’s dolphins were sampled during both time periods. The Hector’s CB 11-12 dataset includes samples collected from Cloudy Bay in 2011 and 2012, for which sampling, genotyping, and identification of 148 individuals are described in Chapter 4. Slightly different sets of loci were used for different analyses presented here, depending on requirements for polymorphism and neutrality, and to aid with direct comparisons between datasets, as indicated in Table 5.1.
5.3.1. Genetic diversity

For each dataset, the number of alleles, expected and observed heterozygosity and departures from Hardy-Weinberg equilibrium, were calculated in GenAlEx v6.5 (Peakall and Smouse 2012). Allelic richness was calculated using the smallest sample size for each locus using the diveRsity package in R v3.0.3 (The R Foundation for Statistical Computing 2012, Keenan et al. 2013). Differences in the number of alleles per locus and allelic richness between datasets were tested using a one-tailed paired Wilcoxon signed rank test, with the hypothesis that the Maui’s dolphin datasets would have fewer alleles than Hector’s dolphins in Cloudy Bay, and would show a decrease over time. Differences in observed and expected heterozygosity per locus between datasets were tested using a one-tailed, paired Student’s t-test, with the hypothesis that the Maui’s dolphin datasets would have lower observed and expected heterozygosity than Hector’s dolphins in Cloudy Bay, and would show a decrease over time. The tests described above, as well as tests for normality and equal variances upon which they were selected, were all implemented in R v3.0.3 (The R Foundation for Statistical Computing 2012). Genetic differentiation between Maui’s and Cloudy Bay Hector’s dolphins and over time for the Maui’s dolphin were also assessed by calculating $F_{ST}$ between the two datasets representing different time periods in ARLEQUIN v.3.5.1.2 (Excoffier and Lischer 2010).

5.3.2. Bottleneck tests

Three tests for bottlenecks were implemented, each taking advantage of different genetic patterns that result from bottlenecks: mode shift, heterozygosity excess and M-ratio. In bottlenecked populations, rare alleles tend to be lost, and a greater proportion of alleles (i.e., mode) will be present at mid-frequencies (Luikart et al. 1998). This causes a mode shift from the typical ‘L’ shaped distribution of a stable population, in which the highest proportion of alleles are present at low frequencies. Mode shifts are transient and can only be detected for a few dozen generations. We examined the shape of the allele
frequency distributions for a mode shift using the 16 loci with sufficient data (>75% individuals) for all three datasets (Table 5.1) to allow direct comparisons between them.

A bottlenecked population will also exhibit heterozygosity excess because rare alleles tend to be lost during a bottleneck, but they have little impact on heterozygosity (Cornuet & Luikart 1996). Therefore, heterozygosity will be higher than expected for a stable population with the same number of alleles. This heterozygosity excess is transient, but will persist for $0.2 - 4N_e$ generations after the bottleneck (Luikart and Cornuet 1998). We tested for heterozygosity excess using a one-tailed Wilcoxon test implemented in BOTTLENECK 1.2.02 (Cornuet and Luikart 1996), which was run assuming three different mutation models: stepwise mutation model (SMM), infinite alleles model (IAM) and the two-phase model (TPM, variance = 30, 70% SMM, 1000 iterations). Each dataset was analyzed using the maximum number of loci with sufficient data (Maui’s 01-07: 23 loci, Maui’s 10-11: 25 loci, Hector’s CB11-12: 20 loci; Table 5.1).

Finally, when a population goes through a bottleneck, the ratio ($M$) of the number of microsatellite alleles to the range of the allele lengths is expected to decrease because alleles will be lost faster than the range of lengths decreases. In a large, stable population, all potential allelic states are expected to be filled, resulting in $M = 1$. The $M$-ratio test can detect bottlenecks that occurred up to 100 or more generations ago. The programs M_P_val and the Critical_M (Garza and Williamson 2001) were used to calculate average $M$ and to identify the critical $M$, or threshold below which the observed value should be considered significant. These analyses require three input parameters: $\theta = 4N_e\mu$, the mutation rate ($\mu$) scaled effective population size ($N_e$) prior to the bottleneck; $p_s$, the proportion of one-step mutations; and $\Delta_g$, the average size of non one-step mutations. As the pre-bottleneck $N_e$ is unknown, $\theta$ values of 1 and 10 were used to represent pre-bottleneck $N_e$ of 500 and 5,000 respectively, assuming $\mu = 5\times10^{-4}$ nucleotides per generation (Goldstein and Schlötterer 1999). Based on a review of empirical literature, Garza and Williamson (2001) recommend $\Delta_g =2.8$ and $p_s =0.88$. A later review by Peery et al. (2012), recommended trying a range of $\Delta_g$ from 2.8 to 3.5 and
from 0.9 to 0.78. Therefore, each dataset was analyzed using input parameters to encompass the minimum and maximum of the recommendations: $\Delta_g = 2.8$, $p_s = 0.9$ and $\Delta_g = 3.5$, $p_s = 0.78$. Monomorphic loci were excluded from this analysis because they can substantially bias results by inflating the $M$-ratio. This analysis was carried out using 17 variable loci to allow direct comparisons between the Maui’s dolphin datasets, and 19 variable loci for Hector’s CB11-12 (Table 5.1).

5.3.3. Inbreeding

The level of inbreeding was evaluated using the population-level inbreeding coefficient ($F_{IS}$; Weir and Cockerham 1984), as well as an individual-based measure of internal relatedness (IR; Amos et al. 2001). $F_{IS}$ measures the mean reduction in observed heterozygosity due to non-random mating in a population. It ranges from -1 to +1, where positive values mean fewer heterozygotes than expected and indicate inbreeding, and negative values mean more heterozygotes than expected, indicating outbreeding.

Average $F_{IS}$ for each population was calculated in ARLEQUIN v.3.5.1.2 (Excoffier and Lischer 2010) using 15 loci with sufficient data for all datasets, excluding PPHO104 which appears to be under selection. Significance was tested by 1000 iterations of permuting gene copies between individuals within a population. Differences in $F_{IS}$ between datasets (polymorphic loci only) were tested using a one-tailed Welch paired t-test, with the hypothesis that the Maui’s dolphin datasets will have higher $F_{IS}$ than Hector’s dolphins in Cloudy Bay, and will show an increase over time.

IR measures the average similarity between the two alleles (one inherited from each parent) that an individual has at each microsatellite locus. Similarity is measured by the number of homozygous loci per individual adjusted by the frequency of the alleles involved. IR is approximately normally distributed around 0, with negative values suggesting relatively ‘outbred’ individuals, values near 0 indicating individuals with ‘unrelated’ parents, and positive values suggesting relatively inbred individuals. IR was calculated with the Excel macro IRmacroN4 developed by Amos (2007), using a consensus set of 13 loci that were variable in all three datasets to allow direct
comparisons. To determine if the observed IR was significantly higher than expected, IR
values were calculated for 1000 populations simulated from the input dataset using
STORM (Frasier 2008).

To assess a change in IR over time for the Maui’s dolphin, the Maui’s 01-07 dataset was
used to establish the baseline allele frequencies for the calculation of IR for both datasets.
The date an individual was first sampled was used to classify it into one of the two time
periods, serving as a proxy for “older” versus “younger” individuals (i.e., an individual
sampled in both 2001 and 2010, would only be represented in the 2001-07 group. An
increase in IR between the two Maui’s dolphin datasets was tested using a one-tailed
paired Wilcoxon signed rank test implemented in the program R v3.0.1 (The R
Foundation for Statistical Computing 2012), with the hypothesis that Maui’s 10-11 will
have a higher IR than Maui’s 01-07.

5.3.4. Simulations
BottleSim v2.6.1 (Kuo and Janzen 2003) was used to simulate the level of diversity
expected for the Maui’s dolphin, assuming that it started with the diversity of Hector’s
dolphins in Cloudy Bay and followed the inferred bottleneck of the Maui’s dolphin. The
Maui’s dolphin bottleneck was approximated in two ways with different starting times
and population sizes. The first, scenario A, started in 1985 with the earliest estimate of
abundance available for the Maui’s dolphin of 140 (Martien et al. 1999). The second,
scenario B, started in 1970 with an abundance of 2000, the approximate pre-decline
abundance extrapolated from population dynamics modeling (Slooten and Dawson 2010).
For both scenarios A and B, the decline was set to reach additional published abundance
estimates at their time of reference (Table 5.1 of Chapter 3), with constant linear declines
between them and ending with 55 individuals in 2011. Given the overlapping time of
sampling for the abundance estimates presented by Baker et al. (2013) and Slooten et al.
(2006), we chose to use the higher precision estimate from Baker et al. as the guide for
the simulated bottlenecks. Simulations were set to generate a population for each year
through 2011, according to the start time and sizes set by the bottleneck and assuming
diploidy, dioecy with random mating, 100% generational overlap (i.e., all individuals started with a random age value, which then determines their reproductive status and mortality for the remainder of their simulated lifetime), longevity of 25, age of reproductive maturity of 7, and an equal sex ratio. Initial genetic diversity was established based on genotypes for 19 loci from Hector’s dolphins in Cloudy Bay (Table 5.1) and 1000 iterations of the simulations were conducted. The number of alleles and observed heterozygosity resulting from the simulations were qualitatively compared to the empirical values for the Maui’s 10-11 dataset.

BottleSim v2.6.1 was also used to simulate the future impact on genetic diversity if the Maui’s dolphin remains at 55 individuals for the next 50 and 100 years (~4 and 8 generations, respectively). This analysis was conducted using the same parameters as above, except the Maui’s 10-11 dataset (25 loci) was used to establish the initial genetic diversity and the bottleneck was held constant at 55 individuals for 100 years.

**5.3.5. Highly diverse locus: PPHO104**

Given the anomalously high diversity observed at locus PPHO104 in Maui’s and Hector’s dolphins (Table 5.1), this locus was sequenced to confirm that it was targeting the intended dinucleotide repeat isolated from the harbor porpoise (*Phocoena phocoena*) (Rosel *et al.* 1999). PPHO104 was amplified for 11 Maui’s dolphins in 10µL reactions containing 1× PCR II buffer, 1.5 mM MgCl2, 0.4 µM each unlabeled primer (Rosel *et al.* 1999), 0.2 mM dNTP, 0.125 units Platinum Taq (Invitrogen) and 10–20 ng/L DNA template. Reactions were carried out using the following thermocycling profile: 93°C for 2 min; (92°C for 30s, 50°C for 45s, 72°C for 50s) × 15; (89°C for 30s, 50°C for 45s, 72°C for 50s) × 20; 72°C for 3 min. Excess nucleotides and primers were removed using ExoSap-IT (USB) before cycle sequencing in both forward and reverse directions with BigDye version 3.1 terminator chemistry (Applied Biosystems, Inc.). Sequence products were cleaned using CleanSEQ (Agentcourt) and run on an ABI 3730 DNA Analyzer. Sequences were aligned and edited using Geneious Pro v5.5.2 (BioMatters).
The potential influence of selection on PPHO104 was investigated using an $F_{ST}$ outlier approach and Ewens-Watterson test for neutrality. LOSITAN (Antao et al. 2008) was used to evaluate the relationship between $F_{ST}$ and expected heterozygosity for each locus and to identify outliers that have excessively high or low $F_{ST}$ compared to the expectation under an island model of migration with neutral markers. To increase the sample size of Maui’s dolphins for this analysis, the two Maui’s dolphin datasets were combined into one with all 86 individuals sampled between 2001 and 2011 (Maui’s 01-11) and compared with Hector’s CB11-12. Both the infinite alleles (IAM) and stepwise (SMM) mutation models were used to run 50,000 simulations with the neutral $F_{ST}$ and forced mean $F_{ST}$ options to identify outlier loci likely to be influenced by selection.

The Ewens-Watterson neutrality test (Ewens 1972, Watterson 1978) compares observed allelic profiles with predictions of neutral theory, assuming the population being tested has maintained a constant size. If the observed homozygosity is lower than predicted, indicating that the frequencies of the $k$ alleles in the sample are more even than expected under neutrality, balancing selection can be inferred. This test was carried out using the C code for the MONTE CARLO program from Slatkin (1996) with 100,000 iterations.

5.4. Results

5.4.1. Genetic diversity

Most microsatellite loci had few alleles, averaging four per locus for each of the Maui’s dolphin datasets and seven alleles/locus for Hector’s CB11-12. An unexpected exception was PPHO104, which had an anomalously high level of diversity and is further explored in the Highly diverse locus: PPHO104 section. As expected, both datasets from the small isolated population of Maui’s dolphins have significantly fewer alleles, lower allelic richness, and lower heterozygosity than Hector’s CB11-12, which represents a larger population thought to be connected to others by gene flow (Hamner et al. 2012; Table 5.1, Table 5.2). Hector’s CB11-12 showed high differentiation from both Maui’s 01-07 ($F_{ST} = 0.14, P < 0.001$) and Maui’s 10-11 ($F_{ST} = 0.15, P < 0.001$). No significant
decrease in the number of alleles, allelic richness, or heterozygosity was detected over the short interval between the two Maui’s dolphin datasets (Table 5.2). The average number of alleles remained at four for both Maui’s 01-07 and Maui’s 10-11, but the observed heterozygosity dropped slightly from 0.328 to 0.315. Additionally, no significant genetic differentiation was found over time between Maui’s 01-07 and Maui’s 10-11, $F_{ST} = -0.005, P = 0.855$.

### 5.4.2. Bottleneck tests

None of the datasets showed a mode shift, as all had the highest peak in the lowest allele frequency class (Figure 5.1). However, both Maui’s datasets had a much less pronounced ‘L’ (i.e., lower total proportion of alleles in the lowest frequency class) when compared to Hector’s CB 11-12 (Figure 5.1), so the Maui’s dolphin could potentially be approaching a mode shift.

The Wilcoxon signed-rank test for heterozygosity excess was sensitive to the mutation model used (Table 5.3). Assuming the infinite allele model (IAM), a significant heterozygote excess was found for Maui’s 01-07 ($P = 0.003$) and Hector’s CB11-12 ($P = 0.004$), and was nearly significant in Maui’s 10-11 ($P = 0.066$). Assuming the two-phase model (TPM), a significant excess was found only for Maui’s 01-07. Assuming the stepwise mutation model (SMM), none of the datasets showed a significant heterozygosity excess (Table 5.3).

Similarly, the $M$-ratio was also sensitive to input parameters related to the mutation model (Table 5.4). The $M$-ratio was highly significant for all three datasets using both values of $\theta$ and the parameters similar to those recommended by Garza and Williamson (2001): $\Delta_g = 2.8, p_s = 0.1$. However, the $M$-ratios were not significant when using the parameters that deviate more from the stepwise mutation model, $\Delta_g = 3.5, p_s = 0.22$, as recommended by Peery et al. (2012). The $M$-ratio for Maui’s 10-11 ($M = 0.768$) showed a slight decrease from Maui’s 01-07 ($M = 0.782$).
5.4.3. Inbreeding

Average $F_{IS}$ did not provide significant evidence for prevalent inbreeding due to non-random mating or for inbreeding avoidance in any of the populations (Table 5.5). Interestingly, Maui’s 01-07 had a lower (i.e., more outbred) average $F_{IS}$ (-0.03) than Hector’s CB11-12 ($F_{IS} = -0.003$), although the difference was not significant (Table 5.2). This pattern could be the result of the Maui’s 01-07 sampling occurring during the phenomenon exploited by the heterozygosity excess test; during a bottleneck, rare alleles are lost rapidly but have little effect on heterozygosity, thus producing a transient excess in heterozygosity relative to that expected in a population of constant size with the same number of alleles (Cornuet and Luikart 1996). This can cause $F_{IS}$ to decrease for a brief period at the onset of a population decline, before it begins to increase. Not surprisingly, Maui’s 10-11 had the highest average $F_{IS} = 0.001$ ($P = 0.527$), although no significant differences were found between any of the datasets (Table 5.1, Table 5.2).

Individuals of all three datasets exhibited a wide range of internal relatedness (IR) values (Table 5.5). Although not directly comparable, due to the different base frequencies used to calculate IR, Cloudy Bay has IR values and range more similar to the earlier Maui’s 01-07 dataset (Table 5.5). Using Maui’s 01-07 as the base population for both Maui’s dolphin datasets, Maui’s 01-07 showed lower IR values on average (mean = -0.019) with more extreme values (range = -0.561 to +0.790) than Maui’s dolphins first sampled in 2010-11 (mean = +0.017; range = -0.286 to +0.446) (Table 5.5; Figure 5.2), although the difference in mean IR between the two time periods was not significant ($t = -0.6681$, df = 81.44, $P = 0.560$).

5.4.4. Simulations

Both bottleneck scenarios A and B produced similar losses of alleles and heterozygosity when applied to the Cloudy Bay Hector’s dolphin genotypes (Table 5.6; Figure 5.3). The empirical data for Maui’s 10-11 had fewer alleles and lower observed heterozygosity than predicted by the simulations for both bottleneck scenarios A and B (Figure 5.3). If the
current Maui’s population (Maui’s10-11) were held constant at 55 individuals for 50 years (~4 generations), the simulations suggest that it would lose 19% of alleles and 3% of observed heterozygosity. After 100 years (~8 generations), it would lose 28% of alleles and 7% of observed heterozygosity (Table 5.5; Figure 5.4).

5.4.5. Highly diverse locus: PPHO104

Sequencing confirmed PPHO104 contained the expected dinucleotide CA repeat microsatellite in Maui’s dolphins, as well as several two to three unit repeats of one or two base pair motifs contained in the flanking sequence (Figure 5.5). These shorter repeats likely contribute to the diversity of PPHO104, and explain the presence of odd and even alleles (Figure 5.6). This locus had a much higher number of alleles than all the other loci, with 36 and 31 alleles in the Maui’s dolphin datasets and 63 alleles in Hector’s CB11-12 (Table 5.1). Almost every individual was heterozygous at this locus, producing very high heterozygosities for all three datasets as well (Table 5.1). Although the Maui’s dolphins sampled represented the majority of the estimated abundances (77% in 2001-07; 73% in 2010-11) and likely captured most of the diversity of PPHO104, the Cloudy Bay sample represents only 54% of this population’s estimated abundance, so it likely has even higher diversity than represented here. Between the two Maui’s dolphin datasets, 5 alleles were lost and observed heterozygosity dropped slightly from 0.958 to 0.950.

LOSITAN identified PPHO104 as being under balancing selection when assuming an infinite allele model, (Figure 5.7a), however, not when assuming a stepwise mutation model (Figure 5.7b). All other loci were found to be within the expectations for neutral loci under an island model of migration using both mutation models. Given the non-stepwise pattern of the alleles observed at PPHO104 (i.e., alleles at most base pairs within the range, despite being a dinucleotide repeat; Figure 5.6), the infinite alleles model is likely more appropriate for this locus. The Ewens-Watterson test provided significant evidence of balancing selection acting on PPHO104 for Maui’s 01-07 and Hector’s CB11-12, however not for the more recent Maui’s 10-11 (Table 5.7).
5.5. Discussion

Despite a lack of historical data on the size and genetic diversity of Maui’s dolphins, it is clear that the current population has reduced evolutionary potential and genetic characteristics that contribute to the risk of extinction. As expected, the small remnant population of Maui’s dolphins had significantly fewer alleles and lower heterozygosity than the more abundant population of its sister subspecies, Hector’s dolphins in Cloudy Bay. Furthermore, the Maui’s dolphin appears to be losing heterozygosity and showing increased levels of inbreeding (\( F_{IS} \) & IR), although these trends were not significant over the short time period between the two datasets 2001-07 and 2010-11. The lack of significance was to be expected given the low power to detect moderate trends in these parameters, especially over such a short time period (<1 generation) for a long-lived animal with overlapping generations (Luikart et al. 1999, Schwartz et al. 2007). However for populations approaching the edge of extinction, it is important to continue some form of monitoring over relatively short periods of time because large changes can occur quickly.

Cumulative evidence suggests a gradual genetic bottleneck for the Maui’s dolphin, despite a lack of significance for some of the bottleneck tests implemented here, which are known to have low power and sensitivity to parameters that are difficult to estimate (e.g., mutation model and rate; Peery et al. 2012). We know that individuals have been lost to anthropogenic causes including incidental fisheries-related mortality (New Zealand Department of Conservation 2014), and that there is currently a very low abundance (Chapter 3, Hamner et al. in press) and effective population size (Chapter 4). The slightly higher \( N_e/N_{f+} \) ratio for Maui’s dolphins over Hector’s dolphins (Chapter 4), is consistent with a recent bottleneck. Linkage disequilibrium can require several generations to decay (Waples 2005, Waples 2006), therefore a large \( N_b/N_{f+} \) could be reflecting information on \( N_e \) from up to a few generations in the past, but \( N_{f+} \) of the present. Furthermore, the empirical data for Maui’s 10-11 showed a greater impact from a bottleneck (i.e., fewer alleles and lower heterozygosity) than predicted by the simulations that started with the diversity of Hector’s dolphins in Cloudy Bay. It is
possible, however, that this discrepancy occurred because our assumption of the initial
diversity or approximated trajectory of the bottleneck does not reflect the reality for the
Maui’s dolphin. It is also possible that the bottleneck may have started earlier than
assumed or that the historical population was smaller than assumed. The Maui’s dolphin
might have historically been a relatively small population with low genetic diversity, as
has been suggested for the vaquita (Rosel and Rojas-Bracho 1999, Taylor and Rojas-
Bracho 1999). Nevertheless, if kept at 55 individuals the genetic diversity of the Maui’s
dolphin will erode over time and inbreeding effects will increase.

Between Maui’s dolphins sampled in 2001-07 and 2010-11, we have documented the loss
of alleles at four neutral microsatellite loci and a decrease in observed heterozygosity at
11 loci. The commonly assumed correlation between neutral and functional genetic
diversity is debatable (e.g., Pearman 2001; Ouborg et al. 2010), but there is also evidence
to suggest that the Maui’s dolphin has experienced a loss of functional diversity. The
Maui’s dolphin has fewer alleles than the Hector’s dolphin at the immunologically
important DQA and DQB genes of the major histocompatibility complex (Heimeier
2009), suggesting a loss of functional diversity at some point since the divergence of the
two subspecies. The loss of functional diversity will limit the subspecies’ ability to adapt
in the short term and constrain its flexibility for adapting to changes over longer
evolutionary timeframes. Maximal retention of genetic diversity at functional loci is
important so that a population can maintain alleles adapted to present conditions, as well
as those adapted to conditions that may occur in the future or periodically (e.g., changes
in food availability, temperature, disease outbreaks, etc.).

As a population declines and fewer reproductively mature and receptive mates are
available, inbreeding is expected to increase, as even random mating in a small
population will lead to some matings between relatives (Keller & Waller 2002). The
observed increase in mean $F_{IS}$ and IR for Maui’s dolphins from 2001-07 to 2010-11
supports this hypothesis, but the increases were small and not statistically significant.
While it is surprising that a population estimated to consist of only 55 (95% CL = 48, 69)
age 1+ individuals does not show stronger evidence of prevalent inbreeding, this finding is consistent with the relatively high effective population size and retention of genetic diversity aided by overlapping generations (*e.g.*, Dinerstein and McCracken 1990, Hailer *et al.* 2006, Lippe *et al.* 2006). However, the trend is concerning as IR has been inversely correlated with fitness (calving success) in long-finned pilot whales (Amos *et al.* 2001) and bottlenose dolphins (Frère *et al.* 2010). Without direct data on calving success, phenotypic expressions of maladapted recessive alleles can also serve as a signal of inbreeding, as seen in some vaquitas with polydactyly, deformed vertebrae, and odd calcifications in the ovaries of adult females (summarized by Taylor and Rojas-Bracho 1999). To my knowledge, no phenotypic indications of inbreeding depression have been documented in Maui’s dolphins. However, the recovery of beachcast Maui’s dolphin dolphins, including pregnant females and neonates that show no signs of entanglement or determinable cause of death (New Zealand Department of Conservation 2014), suggests that perhaps inbreeding might be starting to cause pregnancy/birth-related complications. Alternatively, if the Maui’s dolphin population has historically been small or experienced a gradual decline, it might have been able to purge deleterious alleles, allowing it to adapt to the recent decline in abundance without incurring severe effects of inbreeding depression (Nei *et al.* 1975, Lande 1988, Allendorf *et al.* 2013).

As a relatively philopatric species, it is possible that the Maui’s dolphin might have evolved a mechanism of inbreeding avoidance. Such mechanisms do not always evolve when there is a fitness cost to inbreeding, such as for the bottlenose dolphins in East Shark Bay, Western Australia, which seem to have a higher cost for inbreeding avoidance than tolerance (Frère *et al.* 2010). However, there is evidence for selection of maximally dissimilar mates for the long-finned pilot whale (Amos *et al.* 2001). While the mechanism is unknown, one possibility is major histocompatibility complex (MHC) genes, which are involved with immune response and have been correlated with the selection of mates having maximally dissimilar alleles in a variety of vertebrates (*e.g.*, Milinski 2006). A previous analysis of two MHC loci (*DQA* and *DQB*) showed that although the Maui’s dolphin had only a subset of the alleles (2 alleles at *DQA*; 3 alleles at
found in the Hector’s dolphin populations around the South Island (4 alleles at
DQA; 6 alleles at DQB), the alleles retained by the Maui’s dolphin were the most
divergent ones (Heimeier 2009). This pattern could, however, be the result of a high
differential in survival between individuals with divergent versus similar alleles, as
opposed to driven by mate choice.

The microsatellite locus PPHO104 appears to be influenced by strong diversifying
selection in Hector’s and Maui’s dolphins, and could potentially offer clues to an
inbreeding avoidance mechanism. The high diversity could be the result of strong
heterozygote advantage/deleterious cost to homozygosity or an extremely high mutation
rate, which is likely acting on a functional locus nearby. Microsatellites linked to highly
variable loci, such as MHC genes, are often used as a proxy for measuring the level of
polymorphism of the MHC genes themselves (e.g., Ellegren et al. 1993, Meagher and
Potts 1997, Doxiadis et al. 2007). The anomalously high level of diversity of PPHO104
compared to other loci in Hector’s and Maui’s dolphins was similar to the difference
observed between MHC-linked vs non-MHC linked microsatellites in sheep (Santucci
2007). Very few Maui’s and Hector’s dolphin individuals were homozygous at
PPHO104, suggesting that heterozygosity might offer a great benefit to fitness. If
heterozygotes at this locus, or an adjacent one causing its diversity (not necessarily an
MHC locus), have a far greater probability of surviving than homozygotes, it could be
acting as an inbreeding avoidance mechanism. Interestingly, the Ewens-Watterson test
did not detect significant balancing selection for the most recent Maui’s dolphin dataset
(Maui’s 10-11). This could be a signal that selection is no longer able to act efficiently in
this small population. Alternatively, it could be the result of low sample size or violating
the test’s assumption of constant population size.

There is a relatively high level of genomic conservation across cetaceans from the largest
blue whale to the smallest Hector’s dolphin. This allows them to share microsatellite
primers, which are often species specific in other taxonomic groups (Bourret et al. 2008).
Therefore, it was surprising to discover such a high level of diversity at PPHO104 in
Hector’s and Maui’s dolphins, despite it having a lower level of diversity in the harbor porpoise (*Phocoena phocoena*), from which the locus was characterized (Rosel *et al.* 1999). In each of five populations of harbor porpoise in the northwest Atlantic with sample sizes of 47 to 80 individuals, only 15 to 17 alleles were reported (Rosel *et al.* 1999). Similarly, in a sample of 22 Yangtze finless porpoises (*Neophocaena phocaenoides asiaeorientalis*), only 3 alleles were identified (Xia *et al.* 2005).

Preliminary results indicate that the Chilean dolphin (*Cephalorhynchus eutropia*) has high diversity at this locus (M.J. Perez-Alvarez and R.M. Hamner, unpublished data), so it would be interesting to examine diversity in other cetaceans to see if the high diversity is restricted to the genus *Cephalorhynchus*.

### 5.5.1. Conservation implications

Both Maui’s dolphins and Cloudy Bay Hector’s dolphins are below the recommended threshold $N_e$ ($N_e = 500$, Franklin 1980; $N_e \geq 1000$, Frankham *et al.* 2014) thought to be necessary to preserve long-term evolutionary potential (Chapter 4). This is most concerning for the isolated population of Maui’s dolphins, as the Cloudy Bay Hector’s dolphins are thought to experience some ongoing gene flow with neighboring populations (Hamner *et al.* 2012). Such gene flow should help to facilitate matings between unrelated individuals and maintain genetic diversity in the population over time. The Maui’s dolphin, however, is also below the threshold $N_e$ ($N_e = 50$, Franklin 1980; $N_e \geq 100$, Frankham *et al.* 2014) thought to be necessary to avoid inbreeding depression over the next five generations. Therefore, if the Maui’s dolphin population remains small and isolated, its already low allelic diversity and heterozygosity will continue to decline at a rate of $1/2N_e$ per generation, further reducing the evolutionary potential of the subspecies, increasing the likely effects of inbreeding depression, and thus increasing the risk of extinction.

After experiencing a bottleneck, the recovery of genetic diversity for an isolated population will be slow, as it primarily depends upon the mutation rate (Lynch 1996). However, this process can be accelerated by the immigration of individuals from other
populations resulting in genetic restoration or rescue (e.g., Ingvarsson 2001, Tallmon et al. 2004, Hedrick 2005, Hedrick et al. 2011). Genetic restoration is often accomplished by human intervention in the form of translocating individuals from a closely related population with more or different genetic variation (e.g., Griffith et al. 1989, Moritz 1999, Parker 2008). To be successful, such actions must consider a host of demographic and genetic characteristics of the population of interest and potential effects of translocation (e.g., social structure, behavior, breeding requirements, outbreeding depression, disease introduction, etc.), as well as ensuring that the cause of decline is no longer a threat. However, not all cases of genetic rescue occur as a result of management intervention to translocate individuals. Genetic rescue can also occur from natural dispersal events of as few as one individual per generation, such as those that improved the genetic diversity and fitness of wolves in Scandinavia (Vila et al. 2003) and Isle Royale National Park (Adams et al. 2011). The rare dispersal of Hector’s dolphins from the South Island to the population of Maui’s dolphins was recently documented (Hamner et al. 2014). Although there is currently no evidence of interbreeding between the two subspecies, the natural dispersal of Hector’s dolphins to the North Island provides the potential for enhancing the low genetic diversity of the Maui’s dolphin and preserving the species as part of the west coast North Island ecosystem.

Continued genetic monitoring is important for detecting genetic restoration if it does occur, as well as tracking general changes in genetic diversity. Investigation of the genes adjacent to PPHO104 could provide valuable information about a functionally important gene and potential inbreeding avoidance mechanism. Analysis of additional functionally important loci could also provide more direct insights into the evolutionary potential of Maui’s dolphins and any adaptive differentiation it might have from Hector’s dolphins. Continuing to conduct thorough necropsies of all recovered carcasses (New Zealand Department of Conservation 2014) will also keep us informed of any phenotypic signs of inbreeding depression, and allow us to document the deaths of individuals included in our DNA register.
5.6. Acknowledgments

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5.7. References


Table 5.1. Microsatellite diversity for Maui’s dolphins sampled between 2001 and 2007 (Maui’s 01-07), Maui’s dolphins sampled in 2010 and 2011 (Maui’s 10-11), and Hector’s dolphins sampled in Cloudy Bay in 2011 and 2012 (Hector’s CB11-12). (n = number of individuals, k = number of alleles, AR=allelic richness, \(H_o\) = observed heterozygosity, \(H_e\) = expected heterozygosity, HWE \(P\) = probability of deviation from Hardy-Weinberg equilibrium).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Maui's 01-07</th>
<th>Maui's 10-11</th>
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<tr>
<td></td>
<td>n  k  AR (H_o) (H_e) HWE (P) (F_{IS}) Analyses(^\wedge)</td>
<td>n  k  AR (H_o) (H_e) HWE (P) (F_{IS}) Analyses(^\wedge)</td>
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<td>415/416(^a)</td>
<td>52  2  2  0.327 0.299 0.495 -0.095 2,3</td>
<td>40  2  2  0.350 0.375 0.673 0.067 2,3,6</td>
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<td>EV1(^b)</td>
<td>18  1  1  0.000 0.000 n/a n/a 1</td>
<td>38  1  1  0.000 0.000 n/a n/a 1.2,6</td>
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<td>EV104(^b)</td>
<td>11  1  1  0.000 0.000 n/a n/a 1</td>
<td>39  1  1  0.000 0.000 n/a n/a 2,6</td>
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<td>EV14(^b)</td>
<td>53  3  2.9 0.151 0.237 0.001* 0.364 1,2,3,4,5,7</td>
<td>40  3  3  0.350 0.353 0.965 0.010 1,2,3,4,5,6,7</td>
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<td>EV37(^b)</td>
<td>48  2  2  0.354 0.291 0.136 -0.215 2,3</td>
<td>38  5  4.2 0.289 0.373 &lt;0.001* 0.224 2,3,6</td>
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<td>EV94(^b)</td>
<td>53  3  3  0.604 0.571 0.304 -0.057 1,2,3,4,5,7</td>
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<td>GT211(^c)</td>
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<td>GT575(^c)</td>
<td>53  2  2  0.113 0.107 0.662 -0.060 1,2,3,4,5,7</td>
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<td>KWM12a(^d)</td>
<td>53  6  4.9 0.491 0.497 0.566 0.012 1,2,3,4,5,7</td>
<td>39  7  6.1 0.410 0.460 0.003* 0.109 1,2,3,4,5,6,7</td>
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<td>KWM99b(^c)</td>
<td>53  3  3  0.755 0.612 0.128 -0.234 1,2,3,4,5,7</td>
<td>40  4  3.6 0.725 0.637 0.562 -0.138 1,2,3,4,5,6,7</td>
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<td>MK5(^f)</td>
<td>53  4  3.9 0.585 0.644 0.866 0.092 1,2,3,4,5,7</td>
<td>40  3  3  0.725 0.598 0.087 -0.213 1,2,3,4,5,6,7</td>
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<td>MK6(^f)</td>
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<td>40  2  1.6 0.025 0.025 0.936 -0.013 2,6</td>
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<td>PPHO104(^g)</td>
<td>51  36 27.3 0.941 0.958 0.980 0.017 2.7</td>
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<td>PPHO110(^g)</td>
<td>51  3  2.5 0.490 0.457 0.858 -0.072 1,2,3,4,5,7</td>
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<td>PPHO130(^g)</td>
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<td>PPHO142(^g)</td>
<td>52  2  2  0.538 0.488 0.458 -0.103 1,2,3,4,5,7</td>
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<td>48  1  1  0.000 0.000 n/a n/a 1,2,4,7</td>
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<td>SGUI06(^h)</td>
<td>43  1  1  0.000 0.000 n/a n/a 1,2,4,7</td>
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<td>TexVets(^i)</td>
<td>53  1  1  0.000 0.000 n/a n/a 2</td>
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<td>TriuGT48(^j)</td>
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<td>$F_X$</td>
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<tr>
<td>415/416$^a$</td>
<td>75</td>
<td>3</td>
<td>2.2</td>
<td>0.133</td>
<td>0.125</td>
<td>0.944</td>
<td>-0.065</td>
</tr>
<tr>
<td>EV1$^b$</td>
<td>148</td>
<td>2</td>
<td>2</td>
<td>0.392</td>
<td>0.361</td>
<td>0.300</td>
<td>-0.085</td>
</tr>
<tr>
<td>EV104$^b$</td>
<td>146</td>
<td>3</td>
<td>2.2</td>
<td>0.137</td>
<td>0.163</td>
<td>0.238</td>
<td>0.159</td>
</tr>
<tr>
<td>EV14$^b$</td>
<td>147</td>
<td>4</td>
<td>3.7</td>
<td>0.592</td>
<td>0.675</td>
<td>0.044*</td>
<td>0.123</td>
</tr>
<tr>
<td>EV37$^b$</td>
<td>85</td>
<td>2</td>
<td>2</td>
<td>0.165</td>
<td>0.151</td>
<td>0.408</td>
<td>-0.090</td>
</tr>
<tr>
<td>EV94$^b$</td>
<td>148</td>
<td>9</td>
<td>8.1</td>
<td>0.811</td>
<td>0.742</td>
<td>0.862</td>
<td>-0.093</td>
</tr>
<tr>
<td>GT211$^c$</td>
<td>145</td>
<td>5</td>
<td>4.6</td>
<td>0.710</td>
<td>0.679</td>
<td>0.484</td>
<td>-0.046</td>
</tr>
<tr>
<td>GT23$^c$</td>
<td>141</td>
<td>4</td>
<td>3.5</td>
<td>0.482</td>
<td>0.454</td>
<td>0.675</td>
<td>-0.062</td>
</tr>
<tr>
<td>GT575$^c$</td>
<td>111</td>
<td>3</td>
<td>2.7</td>
<td>0.450</td>
<td>0.383</td>
<td>0.250</td>
<td>-0.176</td>
</tr>
<tr>
<td>KWM12a$^d$</td>
<td>148</td>
<td>24</td>
<td>16.1</td>
<td>0.865</td>
<td>0.863</td>
<td>0.015*</td>
<td>-0.002</td>
</tr>
<tr>
<td>KWM9b$^e$</td>
<td>147</td>
<td>4</td>
<td>4</td>
<td>0.687</td>
<td>0.702</td>
<td>0.237</td>
<td>0.021</td>
</tr>
<tr>
<td>MK5$^f$</td>
<td>148</td>
<td>3</td>
<td>3</td>
<td>0.527</td>
<td>0.592</td>
<td>0.381</td>
<td>0.110</td>
</tr>
<tr>
<td>MK6$^f$</td>
<td>97</td>
<td>6</td>
<td>4.5</td>
<td>0.495</td>
<td>0.503</td>
<td>0.295</td>
<td>0.015</td>
</tr>
<tr>
<td>PPHO104$^g$</td>
<td>144</td>
<td>63</td>
<td>36.5</td>
<td>0.889</td>
<td>0.971</td>
<td>0.049*</td>
<td>0.084</td>
</tr>
<tr>
<td>PPHO110$^g$</td>
<td>146</td>
<td>5</td>
<td>4.3</td>
<td>0.726</td>
<td>0.722</td>
<td>0.030*</td>
<td>-0.006</td>
</tr>
<tr>
<td>PPHO130$^g$</td>
<td>145</td>
<td>6</td>
<td>3.6</td>
<td>0.372</td>
<td>0.379</td>
<td>0.990</td>
<td>0.016</td>
</tr>
<tr>
<td>PPHO142$^g$</td>
<td>147</td>
<td>3</td>
<td>2.2</td>
<td>0.524</td>
<td>0.502</td>
<td>0.755</td>
<td>-0.043</td>
</tr>
<tr>
<td>SGU102$^h$</td>
<td>144</td>
<td>3</td>
<td>2.7</td>
<td>0.063</td>
<td>0.125</td>
<td>&lt;0.001*</td>
<td>0.501</td>
</tr>
<tr>
<td>SGU103$^b$</td>
<td>147</td>
<td>8</td>
<td>7.4</td>
<td>0.810</td>
<td>0.803</td>
<td>0.828</td>
<td>-0.008</td>
</tr>
<tr>
<td>SGU106$^b$</td>
<td>112</td>
<td>4</td>
<td>3</td>
<td>0.518</td>
<td>0.465</td>
<td>0.639</td>
<td>-0.113</td>
</tr>
<tr>
<td>SGU107$^b$</td>
<td>103</td>
<td>2</td>
<td>2</td>
<td>0.466</td>
<td>0.475</td>
<td>0.847</td>
<td>0.019</td>
</tr>
<tr>
<td>SGU111$^b$</td>
<td>146</td>
<td>2</td>
<td>2</td>
<td>0.144</td>
<td>0.133</td>
<td>0.349</td>
<td>-0.077</td>
</tr>
<tr>
<td>SGU116$^b$</td>
<td>148</td>
<td>3</td>
<td>3</td>
<td>0.331</td>
<td>0.310</td>
<td>0.285</td>
<td>-0.070</td>
</tr>
<tr>
<td>SGU117$^b$</td>
<td>147</td>
<td>4</td>
<td>3.2</td>
<td>0.388</td>
<td>0.356</td>
<td>0.767</td>
<td>-0.090</td>
</tr>
<tr>
<td>TexVet5$^i$</td>
<td>41</td>
<td>4</td>
<td>3.1</td>
<td>0.732</td>
<td>0.564</td>
<td>0.384</td>
<td>-0.298</td>
</tr>
<tr>
<td>TtruGT48$^i$</td>
<td>95</td>
<td>6</td>
<td>4.9</td>
<td>0.589</td>
<td>0.663</td>
<td>0.004*</td>
<td>0.111</td>
</tr>
<tr>
<td>Overall Ave.</td>
<td>148</td>
<td>7</td>
<td>5.2</td>
<td>0.500</td>
<td>0.495</td>
<td>-0.006</td>
<td></td>
</tr>
</tbody>
</table>


$^1$Mode shift, $^2$Heterozygosity excess, $^3$M-ratio, $^4$F$_{IS}$, $^5$IR, $^6$BottleSim, $^7$Lostian
Table 5.2. Tests for differences in alleles ($k$), allelic richness (AR), observed ($H_o$) and expected ($H_e$) heterozygosity, and $F_{is}$ between Maui’s dolphins sampled between 2001 and 2007 (Maui’s 01-07), Maui’s dolphins sampled in 2010 and 2011 (Maui's 10-11), and Hector’s dolphins sampled in Cloudy Bay in 2011 and 2012 (Hector's CB11-12). An increase in Internal relatedness (IR) as also tested between the two Maui’s datasets, using Maui’s 01-07 as the base population for the calculation of IR for both. See Table 5.1 for locus specific and average values of the first five parameters and Table 5.5 for average values of IR (df = degrees of freedom, $P < 0.001*$).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test</th>
<th>Comparison</th>
<th>Test Statistic</th>
<th>df</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k$</td>
<td>Wilcoxon paired signed rank test</td>
<td>Maui's 01-07 - Maui's 10-11</td>
<td>$V = 24.5$</td>
<td>0.798</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maui's 01-07 - Hector's CB11-12</td>
<td>$V = 295.0$</td>
<td>0.001*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maui's 10-11 - Hector's CB11-12</td>
<td>$V = 260.5$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AR</td>
<td>Wilcoxon paired signed rank test</td>
<td>Maui's 01-07 - Maui's 10-11</td>
<td>$V = 28$</td>
<td>0.817</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maui's 01-07 - Hector's CB11-12</td>
<td>$V = 295$</td>
<td>0.001*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maui's 10-11 - Hector's CB11-12</td>
<td>$V = 283$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$H_o$</td>
<td>Student's paired t-test</td>
<td>Maui's 01-07 - Maui's 10-11</td>
<td>$t = 0.818$</td>
<td>25</td>
<td>0.211</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maui's 01-07 - Hector's CB11-12</td>
<td>$t = 3.620$</td>
<td>25</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maui's 10-11 - Hector's CB11-12</td>
<td>$t = 4.039$</td>
<td>25</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>$H_e$</td>
<td>Student's paired t-test</td>
<td>Maui's 01-07 - Maui's 10-11</td>
<td>$t = -0.368$</td>
<td>25</td>
<td>0.642</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maui's 01-07 - Hector's CB11-12</td>
<td>$t = 4.230$</td>
<td>25</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maui's 10-11 - Hector's CB11-12</td>
<td>$t = 4.145$</td>
<td>25</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>$F_{is}$</td>
<td>Welch paired t-test</td>
<td>Maui's 01-07 - Maui's 10-11</td>
<td>$t = -1.067$</td>
<td>18</td>
<td>0.850</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maui's 01-07 - Hector's CB11-12</td>
<td>$t = 0.794$</td>
<td>18</td>
<td>0.219</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Maui's 10-11 - Hector's CB11-12</td>
<td>$t = -0.660$</td>
<td>18</td>
<td>0.741</td>
</tr>
<tr>
<td>IR</td>
<td>Welch t-test</td>
<td>Maui’s 01-07 - Maui’s 10-11</td>
<td>$t = -0.668$</td>
<td>81</td>
<td>0.506</td>
</tr>
</tbody>
</table>
Table 5.3. Probabilities of heterozygosity excess (*\( P < 0.05 \)) in Maui’s dolphins sampled between 2001 and 2007 (Maui’s 01-07), Maui’s dolphins sampled in 2010 and 2011 (Maui’s 10-11), and Hector’s dolphins sampled in Cloudy Bay in 2011 and 2012 (Hector’s CB11-12) based on a one-tailed Wilcoxon signed rank test in BOTTLENECK 1.2.02 for three assumed mutation models. The two-phased model was 70% stepwise mutation model with variance of 30.

<table>
<thead>
<tr>
<th></th>
<th>Maui’s 01-07</th>
<th>Maui’s 10-11</th>
<th>Hector’s CB11-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>53</td>
<td>40</td>
<td>148</td>
</tr>
<tr>
<td>Number of loci</td>
<td>17</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Infinite alleles model</td>
<td>0.003*</td>
<td>0.066</td>
<td>0.004*</td>
</tr>
<tr>
<td>Two-phased model</td>
<td>0.037*</td>
<td>0.351</td>
<td>0.271</td>
</tr>
<tr>
<td>Stepwise mutation model</td>
<td>0.196</td>
<td>0.715</td>
<td>0.909</td>
</tr>
</tbody>
</table>

Table 5.4. The average ratio (\( M \)) of the number of microsatellite alleles to their length range in Maui’s dolphins sampled between 2001 and 2007 (Maui’s 01-07), Maui’s dolphins sampled in 2010 and 2011 (Maui’s 10-11), and Hector’s dolphins sampled in Cloudy Bay in 2011 and 2012 (Hector’s CB11-12). Tests were run using several values for the mutation rate (\( \mu \)) scaled effective population size (\( N_e \)) (\( \theta = 4N_e\mu \)), average size of non-one-step mutations (\( \Delta_g \)), proportion of non-one-step mutations (\( 1-p_s \)). *Indicates a significant bottleneck (\( P < 0.05 \), Average \( M < \) critical \( M_c \)).

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Average ( M )</th>
<th>( \theta )</th>
<th>( \Delta_g )</th>
<th>( 1-p_s )</th>
<th>( P )</th>
<th>( M_c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maui’s 01-07</td>
<td>0.782</td>
<td>1</td>
<td>2.8</td>
<td>0.1</td>
<td>&lt;0.001*</td>
<td>0.849*</td>
</tr>
<tr>
<td>( n = 53 )</td>
<td></td>
<td>10</td>
<td>2.8</td>
<td>0.1</td>
<td>0.005*</td>
<td>0.794*</td>
</tr>
<tr>
<td>17 loci</td>
<td></td>
<td>1</td>
<td>3.5</td>
<td>0.22</td>
<td>0.222</td>
<td>0.710</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>3.5</td>
<td>0.22</td>
<td>0.825</td>
<td>0.659</td>
</tr>
<tr>
<td>Maui’s 10-11</td>
<td>0.768</td>
<td>1</td>
<td>2.8</td>
<td>0.1</td>
<td>&lt;0.001*</td>
<td>0.849*</td>
</tr>
<tr>
<td>( n = 40 )</td>
<td></td>
<td>10</td>
<td>2.8</td>
<td>0.1</td>
<td>0.018*</td>
<td>0.786*</td>
</tr>
<tr>
<td>17 loci</td>
<td></td>
<td>1</td>
<td>3.5</td>
<td>0.22</td>
<td>0.277</td>
<td>0.713</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>3.5</td>
<td>0.22</td>
<td>0.946</td>
<td>0.648</td>
</tr>
<tr>
<td>Hector’s CB11-12</td>
<td>0.758</td>
<td>1</td>
<td>2.8</td>
<td>0.1</td>
<td>&lt;0.001*</td>
<td>0.859*</td>
</tr>
<tr>
<td>( n = 148 )</td>
<td></td>
<td>10</td>
<td>2.8</td>
<td>0.1</td>
<td>&lt;0.001*</td>
<td>0.829*</td>
</tr>
<tr>
<td>19 loci</td>
<td></td>
<td>1</td>
<td>3.5</td>
<td>0.22</td>
<td>0.205</td>
<td>0.715</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>3.5</td>
<td>0.22</td>
<td>0.449</td>
<td>0.706</td>
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</tbody>
</table>
Table 5.5. Average internal relatedness (IR) and coefficient of inbreeding due to non-random mating ($F_{IS}$) for Maui’s dolphins sampled between 2001 and 2007 (Maui’s 01-07), Maui’s dolphins sampled in 2010 and 2011 (Maui’s 10-11), and Hector’s dolphins sampled in Cloudy Bay in 2011 and 2012 (Hector’s CB11-12).

<table>
<thead>
<tr>
<th></th>
<th>Maui's 01-07</th>
<th>Maui's 10-11</th>
<th>Hector’s CB 11-12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>53</td>
<td>40</td>
<td>148</td>
</tr>
<tr>
<td><strong>Average IR</strong></td>
<td>-0.019</td>
<td>0.017</td>
<td>-0.007</td>
</tr>
<tr>
<td><strong>IR range</strong></td>
<td>-0.561 to +0.790</td>
<td>-0.286 to +0.446</td>
<td>-0.494 to +0.571</td>
</tr>
<tr>
<td><strong>IR P</strong></td>
<td>0.693</td>
<td>0.521</td>
<td>0.811</td>
</tr>
<tr>
<td><strong>Average $F_{IS}$</strong></td>
<td>-0.03</td>
<td>0.001</td>
<td>-0.003</td>
</tr>
<tr>
<td><strong>$F_{IS}$ P</strong></td>
<td>0.783</td>
<td>0.527</td>
<td>0.585</td>
</tr>
</tbody>
</table>

Table 5.6. Average number of alleles ($k$) and observed heterozygosity ($H_o$) expected for two simulation scenarios: (I) assuming the initial genetic diversity of Cloudy Bay Hector’s dolphins (19 microsatellites) and the approximate bottleneck of the Maui’s dolphin from an initial abundance of either 140 individuals in 1985 (bottleneck A) or 2000 individuals in 1970 (bottleneck B); and (II) assuming the initial genetic diversity for Maui’s dolphins in 2010-11 (25 microsatellites) and a constant population size of 55 individuals for 50 and 100 years.

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial diversity</td>
<td>Hector’s CB11-12</td>
<td>Maui's10-11</td>
</tr>
<tr>
<td>Initial abundance</td>
<td>140</td>
<td>2000</td>
</tr>
<tr>
<td>Bottleneck scenario</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Years Simulated</td>
<td>27</td>
<td>42</td>
</tr>
<tr>
<td><strong>Average $k$</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start</td>
<td>9.3</td>
<td>10.7</td>
</tr>
<tr>
<td>End</td>
<td>5.9</td>
<td>5.8</td>
</tr>
<tr>
<td>% Lost</td>
<td>36</td>
<td>46</td>
</tr>
<tr>
<td><strong>Average $H_o$</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start</td>
<td>0.54</td>
<td>0.54</td>
</tr>
<tr>
<td>End</td>
<td>0.53</td>
<td>0.53</td>
</tr>
<tr>
<td>% Lost</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 5.7. Ewens-Watterson test for neutrality of microsatellite locus PPHO104 for Maui’s dolphins sampled between 2001 and 2007 (Maui’s 01-07), Maui’s dolphins sampled in 2010 and 2011 (Maui’s 10-11), and Hector’s dolphins sampled in Cloudy Bay in 2011 and 2012 (Hector’s CB11-12). (n = sample size, k = number of alleles, \( \theta = 4N_e\mu \), \( F = \) probability that two genes drawn at random are of the same allelic type, \( P < 0.5^* \))

<table>
<thead>
<tr>
<th></th>
<th>2n</th>
<th>k</th>
<th>( \theta )</th>
<th>( F )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maui’s 01-07</td>
<td>102</td>
<td>36</td>
<td>19.4</td>
<td>0.042</td>
<td>0.025*</td>
</tr>
<tr>
<td>Maui’s 10-11</td>
<td>80</td>
<td>31</td>
<td>18.1</td>
<td>0.053</td>
<td>0.217</td>
</tr>
<tr>
<td>Hector’s CB11-12</td>
<td>270</td>
<td>63</td>
<td>25.5</td>
<td>0.031</td>
<td>0.048*</td>
</tr>
</tbody>
</table>
Figure 5.1. Allele frequency distributions of 16 microsatellites for (a) Maui’s dolphins sampled between 2001 and 2007, (b) Maui’s dolphins sampled in 2010 and 2011, and (c) Hector’s dolphins sampled in Cloudy Bay in 2011 and 2012 (note change in y-axis scale).
Figure 5.2. Internal relatedness of Maui's dolphins sampled between 2001 and 2011, using genotypes for individuals sampled between through 2007 (left of gray dashed line) as the reference population for the calculations. Seven individuals (^ or v) sampled during both time periods are only represented in the earlier dataset to the left of the line.
Figure 5.3. Temporal progression of the average number of alleles (a, c) and observed heterozygosity ($H_o$; b, d) simulated assuming the initial genetic diversity of Cloudy Bay Hector’s dolphins (19 microsatellite loci) and the approximate bottleneck of the Maui’s dolphin from an initial abundance of either 140 individuals in 1985 (Scenario A; a, b) or 2000 individuals in 1970 (Scenario B; c, d).
Figure 5.4. Predicted (a) average number of alleles and (b) observed heterozygosity ($H_o$) for the Maui’s dolphin based on simulations starting with the diversity of Maui’s dolphins sampled in 2010 and 2011 and running for 100 years (~8 generations) into the future.
Figure 5.5. Sequences of PPHO104 from 10 Maui’s dolphins aligned with the harbor porpoise (at top; Rosel et al. 1999), confirming the expected dinucleotide CA repeat, as well as showing other short repeats in the flanking sequence. The Maui’s dolphins were sequenced using the reverse primer. Visualization exported from Geneious 6.1, with length indicated in base pairs from the 5’ end of the harbor porpoise sequence shown.
Figure 5.6. Allele frequency distributions for anomalously diverse microsatellite locus PPHO104 for (a) Maui’s dolphins sampled between 2001 and 2007, (b) Maui’s dolphins sampled in 2010 and 2011, and (c) Hector’s dolphins sampled in Cloudy Bay in 2011 and 2012. The sample size of alleles ($2n$) and number of different alleles ($k$) are indicated for each dataset.
Figure 5.7. $F_{ST}$ outlier analysis between Maui’s dolphin individuals sampled between 2001 and 2011 ($n = 86$) and Hector’s dolphin individuals sampled in Cloudy Bay in 2011 and 2012 ($n = 148$), run in LOSITAN assuming an (a) infinite allele model and (b) stepwise mutation model.
6. General Discussion

Optimal strategies for conservation and management decisions emerge from considerations that include the most complete understanding of a taxon’s biology and ecology. In 2007, the Hector’s and Maui’s Dolphin Threat Management Plan identified distribution, abundance, gene flow, and life history characteristics as the top four research priorities for these subspecies (New Zealand Department of Conservation and Ministry of Fisheries 2007). The goal of my research was to use genetic tools to examine a range of demographic and genetic parameters relevant for conservation considerations regarding Maui’s and Hector’s dolphins, which contributed to three of these research priorities. The results presented here provide information on the abundance, effective population size, and genetic diversity of the critically endangered Maui’s dolphin, using the endangered Hector’s dolphin population in Cloudy Bay for comparison, and document the unexpected dispersal of Hector’s dolphins to the North Island.

The critically low abundance of Maui’s dolphins was confirmed using genotype recapture analysis, which provided higher precision than past estimates (Chapter 3, Hamner et al. in press). At approximately 55 individuals age 1+, the Maui’s dolphin has the lowest abundance estimated to date for any cetacean species or subspecies listed by the International Union for Conservation of Nature (IUCN; Appendix I). A DNA register of Maui’s dolphins known to be alive at the time of our sampling (2010-2011) includes 71% \((n = 39)\) of the estimated 55 individuals remaining. Additionally, the genotypes were used to estimate the effective population size of the parental generation to be \(N_e = 61\), using the linkage disequilibrium method (Chapter 4). By using the abundance estimate for approximately 2003 \((N_{1+} = 69;\) Baker et al. 2013; Appendix I) to minimize bias due to a generational lag, the approximate \(N_e/N_{1+}\) ratio is 0.88. This high ratio is promising for the evolutionary potential of the Maui’s dolphin, but the current \(N_e\) of approximately 49 is still lower than the recommended thresholds thought to be necessary to avoid inbreeding depression over five generations \((N_e = 50,\) recently increased to \(\geq 100\)) and to preserve long-term evolutionary potential in perpetuity \((N_e = 500,\) recently increased to \(\geq 1000;\) Franklin 1980; Frankham et al. 2014).
Preliminary genetic monitoring of the Maui’s dolphin was initiated by comparing data from the individuals sampled in 2001-07 and 2010-11. These data suggest a decline in abundance, effective population size, and heterozygosity, and an increase in inbreeding ($F_{IS}$ and internal relatedness) over this time period, although none of the differences were statistically significant (Chapter 5, Appendix VI). The lack of significance was to be expected given the low power to detect moderate trends in these parameters over such a short time period (<1 generation) for a long-lived animal with overlapping generations (Taylor and Gerrodette 1993, Luikart et al. 1999, Schwartz et al. 2007, Taylor et al. 2007). However, for such a small population it is important to have some form of monitoring at shorter intervals than would be recommended to achieve power to detect a trend. As a population declines, so does the power to detect trends (Taylor and Gerrodette 1993), but with only about 55 individuals remaining, large changes can take place over relatively short periods of time. If monitoring intervals are too long, it may be too late for management action by the time a change is detected. Therefore, despite the low power to detect significant trends, the results presented here serve as valuable data points for the long-term monitoring of this subspecies.

In contrast to the Maui’s dolphin, an estimated 272 Hector’s dolphins inhabit Cloudy Bay (Chapter 4) – an area approximately seven times smaller than the area over which the remaining Maui’s dolphins were sighted (Appendix III, Oremus et al. 2012). This population of Hector’s dolphins also has a higher $N_e$ estimated to be 207 using the linkage disequilibrium method, and significantly higher average number of alleles/locus and heterozygosity. Although this population has a relatively low abundance and $N_e$ compared to many other species, it is thought to be linked to adjacent populations by gene flow (Hamner et al. 2012), which will help it to maintain genetic diversity and evolutionary potential. Thus, the isolated Maui’s dolphin is more vulnerable to losing genetic diversity and currently has a lower evolutionary potential and higher risk of extinction, compared to Hector’s dolphins in Cloudy Bay.
The isolation of the Maui’s dolphin has been interrupted by what seems to be a very rare dispersal event of Hector’s dolphins (Chapter 1). The unexpected identification of two female Hector’s dolphins living among the Maui’s dolphins provides the potential for genetic restoration in Maui’s dolphins, if interbreeding occurs. Even as few as one immigrant establishing gene flow can have profound genetic impacts on a small population with low genetic diversity (e.g., Vila et al. 2003, Adams et al. 2011). At the time of our sampling, there was no evidence of interbreeding (gene flow) between the subspecies. It may be some time before evidence is available for detection, as the ages of the immigrants are unknown and these dolphins do not reach sexual maturity until they are 6 to 9 years old (Slooten and Lad 1991).

Our findings are the first contemporary evidence of Hector’s and Maui’s dolphins co-occurring in the same area. Although four Hector’s dolphins have now been documented within the geographic range of the Maui’s dolphin, it is premature to raise concerns about the validity of the subspecies. To date, we have not detected evidence of interbreeding between the Hector’s and Maui’s dolphins, and there are no examples from captivity to assess this potential. The number of documented dispersal events at this time is low. However, if further dispersal of Hector’s dolphins occurs and the subspecies are shown to interbreed, it could lead to a loss of the genetic and morphological distinctiveness that was used to support their classification as subspecies (Reeves et al. 2004, Perrin et al. 2009).

The unexpected dispersal of Hector’s dolphins to the North Island was surprising for several reasons given previous research on Hector’s and Maui’s dolphins. The identification of two Hector’s dolphins from the west coast of the South Island sampled on the North Island represents the longest distance over which individuals have been documented traveling for this species (≥400km). Despite over 25 years of previous research on Hector’s dolphins, the maximum distance of dispersal observed was just over 100 km, with most observed movements within a home range of 30 to 60 km (Bräger et al. 2002, Rayment et al. 2009). The long-distance dispersal also suggests that there is a
corridor connecting the North and South Islands. The deep water of Cook Strait was thought to deter these dolphins from moving between the islands, consistent with most observations of Hector’s dolphins occurring in depths less than 39 m (Bräger et al. 2003, Rayment et al. 2011) and the rarity of sightings in the Fiordland area where depths can exceed 300 m (Cawthorn 1988). It is not known whether the dispersing dolphins crossed the deeper waters at the narrowest point of Cook Strait, or perhaps followed an offshore corridor of shallower water to the northwest.

Four of the six Hector’s dolphins sampled on the North Island did not clearly assign to one of the three regional populations (Chapter 1). This suggests the potential for a previously unsampled population that is not included in our baseline reference data, or perhaps an area of interbreeding between the East and West Coast Hector’s dolphin populations. Therefore, there is the potential for a small and elusive resident population of Hector’s dolphins along the southern part of the North Island, outside the current range of the Maui’s dolphin, or along the northern part of the South Island between the East and West Coast populations of Hector’s dolphins.

6.1. Conservation Implications and Management Actions

Despite its very low abundance, effective population size, and genetic diversity, the Maui’s dolphin should not necessarily be considered doomed to extinction. The population appears to be maintaining an equal sex ratio and connectivity across the remaining distribution by individual movements up to 80 km (Appendix III, Oremus et al. 2012). Although its current $N_e$ has declined below the recommended thresholds for avoiding inbreeding depression, there is hope for survival if the decline can be halted and the population is allowed to recover at its intrinsic rate of increase (Frankham et al. 2014). Although there is currently no evidence of interbreeding between the two subspecies, the documentation of Hector’s dolphins naturally dispersing to the North Island (Chapter 1, Hamner et al. 2014) provides the potential for enhancing the low genetic diversity and evolutionary potential of the Maui’s dolphin, and preserving the
species as part of the west coast North Island ecosystem. If protected corridors connecting the Maui’s dolphin on the North Island and Hector’s dolphin populations on the South Island are not maintained, then such natural dispersal events are less likely to occur.

To give Maui’s dolphins the best chance of long-term survival, anthropogenic mortality must be minimized to below the potential biological removal threshold of one dolphin every 10 to 23 years (Chapter 3, Hamner et al. in press). Barring interference from stochastic factors, the Maui’s dolphin could then start the long, slow process of recovery. Their maximum population growth rate is estimated to be 4.9% per year, assuming a 95% non-calf survival rate and optimal population growth parameters (Slooten and Lad 1991). However, Slooten and Lad (1991) reported that a realistic range for the population growth rate is 1.8 - 4.4%, and suggest that 2.2% per year is the most likely rate. Assuming that Maui’s dolphins were able to recover at a rate of 2.2% per year, it would take 32 years for the population to double in abundance from the current 55. Maximizing the rate of recovery will promote maximum retention of evolutionary potential (Caughley 1994) and give Maui’s dolphins the best chance for long-term survival in a changing environment.

The results presented in Chapters 2 and 3 were reported first to the New Zealand government, and contributed to accelerating the review of the Maui’s Dolphin Threat Management Plan (New Zealand Minstry for Primary Industries and Department of Conservation 2012). The deaths of two dolphins in 2012 near Cape Egmont – south of the set net and trawling bans implemented in 2008 – confirmed the presence of Hector’s (Hamner et al. 2014) and perhaps Maui’s dolphins in this area. These deaths led to an extension of commercial and recreational set net restrictions out to 7nm from the former boundary at Pariokariwa Point south to the Waiwhakaiho River, and extending further south to Hawera out to 2 nm from shore with observers required for commercial set netting between 2 and 7 nm (New Zealand Ministry for Primary Industries 2012, New Zealand Department of Conservation and Minstry for Primary Industries 2013; Figure
3.1). This decision will reduce entanglement risk to Maui’s dolphins utilizing the southern part of their distribution, as well as any Hector’s dolphins that disperse north into that area. Our findings reported for the population of Hector’s dolphins in Cloudy Bay provide information that will contribute to the upcoming review of the Hector’s dolphin component of the Threat Management Plan.

6.2. Future Research and Genetic Monitoring

The genetic approach for answering both demographic and genetic questions was used to maximize the information gained from limited resources and minimal interaction with the dolphins, and with genetic monitoring as a long-term goal. Logistical and financial challenges have meant that the use of genetic monitoring for marine mammals is not as widespread as it is for terrestrial or fish species (Jackson et al. in review). My PhD research demonstrates that genetic monitoring studies are feasible for at least some cetaceans and can provide a wealth of knowledge necessary for management decisions in a timeframe useful for influencing policy.

The continued genetic monitoring of Maui’s and Hector’s dolphins is important for tracking changes in the parameters for which a baseline has been established (e.g., abundance, effective population size and genetic diversity) and gauging threats from inbreeding. To facilitate such work, tissue and DNA from all Maui’s and Hector’s dolphin samples (of sufficient size) that were collected and utilized by my research are archived in the New Zealand Cetacean Tissue Archive at the University of Auckland (Thompson et al. 2013), and the resulting genetic datasets are archived at the Cetacean Conservation and Genetics Lab, Oregon State University (Appendix V). If the two living female Hector’s dolphin immigrants breed with Maui’s dolphins, their relative breeding success can be tracked by monitoring the frequencies of their distinctive maternally-inherited mtDNA haplotypes. Additionally, biparentally-inherited microsatellite genotypes can be used to detect potential evidence of admixture between the subspecies and genetic restoration of the Maui’s dolphin. The recovery of Maui’s dolphin carcasses,
including pregnant females and neonates, that show no signs of entanglement or determinable cause of death (New Zealand Department of Conservation 2014) suggests that inbreeding effects may be starting to adversely affect the small population. Therefore, in addition to monitoring genetic measures of inbreeding, continuing to conduct thorough necropsies of all recovered carcasses (New Zealand Department of Conservation 2014) will aid in identifying potential phenotypic signs of inbreeding depression, and allow us to document the deaths of individuals included in the DNA register.

Genetic monitoring should also be carried out for Hector’s dolphins as well, to identify particularly at risk populations before they decline to the critical state of the Maui’s dolphin. The possibility of a small and elusive population of Hector’s dolphins along the southern part of the North Island or along the northern part of the South Island between the East and West Coast populations should be investigated. Samples from the occasional sightings in these areas will allow us to determine if there is genetically differentiated resident population in these areas, or if it is an area of mixing between individuals from the East and West Coast South Island populations. In either case, the dolphins in these areas might represent an important link for potential gene flow with Maui’s dolphins.

The addition of functionally important loci to monitoring plans could provide more direct insights into the evolutionary potential of Maui’s dolphins, as well as any adaptive differentiation they might have from Hector’s dolphins. For example, monitoring diversity at several major histocompatibility complex (MHC) loci, for which a baseline has been established (Heimeier 2009), would provide insight into changes in the ability to initiate an immune response. Correlations could then be examined between particular genotypes or internal relatedness and infectious disease-related causes of death (e.g., Roe et al. 2013). Identification of the genes adjacent to PPHO104 will likely reveal a functionally important gene, not necessarily MHC, which could potentially be acting as an inbreeding avoidance mechanism. Additionally, the comparison of reduced-
representation genome scans (e.g., RAD-seq, Baird et al. 2008) between populations could be used to identify $F_{ST}$ outlier loci, which can then be investigated as candidates for adaptive differentiation. This information would help to identify the unique evolutionary variation (or relative proportion of it) that would be lost if the Maui’s dolphin goes extinct. It could also be used to select the most appropriate source populations, minimizing the potential for outbreeding depression, if translocation were to be considered. Expansions of the genetic monitoring scheme can easily be made to include more loci or genome scans and extended back to include previously analyzed samples, as tissue and DNA were archived as part of my work (Appendix V). Recent technological advances are rapidly transforming conservation genetics into conservation genomics (Allendorf et al. 2010), which is bringing the cost and timeframe to complete larger scale genomic studies to within the typically tight budget and timeframe required for conservation studies and management decisions.

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Appendices
IUCN

(EN)
EN
(EN)

(EN)
(EN)
EN
(EN)

(EN)
CR
VU
EN
EN
EN

B. b. schlegellii
Balaenoptera physalus
B. p. physalus

B. p. quoyi
B. p. patachonica
Balaenoptera musculus
B. m. musculus

B. m. indica
B. m. intermedia
Physeter macrocephalus
Platanista gangetica
P. g. gangetica
P. g. minor

EXe
VU

EN
(EN)
CR
VU

EN
VU
VU

VU

Lipotes vexillifer
Pontoporia blainvillei

Cephalorhynchus hectori
C. h. hectori
C. h. maui
Sousa teuszii

Tursiops truncatus ponticus
Stenella longirostris orientalis
Delphinus delphis ponticus

Orcaella brevirostris

CR;
Probably

EN
(EN)

Statusb
EN
EN

Balaenoptera borealis
B. b. borealis

Species or Subspeciesa
Eubalaena glacialis
Eubalaena japonica

2008
2001
1991-1997
≤2008

global
central & NE Atlantic
eastern North Pacific Ocean (Washington to Baja California)
Eastern South Pacific

South Island, New Zealand
West Coast North Island, New Zealand
Total distribution
Dahkla Bay, Morocco
Banc d' Arguin, Mauritania
Saloum River delta, Senegal
Guinea Bissau
Flamingos, Angola
NW, N and NE Black Sea in Ukrainian and Russian territorial waters
eastern tropical Pacific
total
Turkish Straits System (Bosphorus, Marmara Sea and Dardanelles)
NW, N and NE Black Sea in Ukrainian and Russian territorial waters
Central Black Sea beyond territorial waters of Ukraine and Turkey
SE Black Sea within Georgian territorial waters
Chilka Lagoon, India
Sundarbans Delta, Bangladesh
coastal waters of Bangladesh
Ayeyarwady River, Myanmar

Rio Grande do Sul, Brazil & Uruguay
Rio Grande do Sul
coast of São Paulo, Paraná and Santa Catarina
coast of Rio de Janeiro and Espirito Santo, Brazil

Ganges-Brahmaputra-Megna and Karnaphuli-Sangu river systems
Indus River system (partial)
Indus River system

1998-2001
2010-2011
2008
1996
≤2004
≤2004
≤2004
2008
2003
2003
≤2005
1998
2003
2005
2003
2003-2006
2002
2004
2003-2004

2006
1996
1996
2008/2009
2011/2012

1982-2004
2006
2001/2006

1997/98
2002

1999-2003
1996-2001
2002
~2000
1999
2002-3
2005
1996-2001
2001
1989
1991
1997

Bering Sea and Alaska Peninsula
west coast of USA
Hawaii
North Atlantic
east coast of North America, Georges Bank to Gulf of St Lawrence
Newfoundland
West Greenland
northeastern North Atlantic
central North Atlantic
North Atlantic: Spain-Portugal-British Isles area
western Mediterranean Sea
Southern hemisphere

south of 60ºS latitude
global

1989
1989
2005
2005
1991-2005
1974
1978-1988

1998
1998-2008
1998-2004
≤2001

Year(s)

North Atlantic: Icelandic waters
North Atlantic; Faroese waters
North Atlantic: East Greenland
North Atlantic: West Greenland
North Pacific: west coast USA
North Pacific
Southern hemisphere

east coast North America
eastern North Pacific
eastern North Pacific
western North Pacific

Population or Area Estimated

33,047
225
4,434
796

1312

1,160

53,542
364
16,390
5,013

7014

4,500

1,419

28,920
6,027

10,391
2,130

358

654

17,227
443
2,465
3,705
227

n/a
54
42

459

6,048
39
236
690
15

n/a
23
24

95% CL
Lower Upper

7270
5303
9966
55
48
69
probably a few thousand
28
high hundreds
~100
several hundred
10
4193
2527
6956
612,662
374,055 868,732
10,000s, possibly ≥100,000
994
390
2,531
5,376
2,898
9,972
4,779
1,433 15,945
9,708
5,009 18,814
85 (range 62-98)
451
337
565
5,383
2,385 12,150
59-72

none detected
42,078
286
8,525
1,998

1,200–1,800
1442
1550-1750

5,600
3,279
174
53,000
2,814
1,013
1,722
4,100
25,800
17,355
3,583
38,185
no estimate available
plausibly 10,000-25,000
855
2,994
~1,000 whales is implied
no estimate available
2,280
361,400

10,207
132
763
1,599
98
8600d
9,718

Nc
299 (max 437)
31
28
low to mid hundreds

0.096
0.395

0.219

0.162
0.15

0.3447
0.34
0.48

0.572

0.36
0.36

0.353
0.14

0.37
0.21
0.125
0.27
0.27

0.21

0.31
0.72

0.272
0.685
0.47
0.42
0.57

CV

vessel and aerial line-transect surveys
genotype recapture
"rough estimate" based on those below & extrapolations
aggregated number of dolphins observed from 4 sightings
"rough estimate"
"rough estimate"
"rough estimate"
vessel- and shore-based surveys; census of photo-identified individuals
vessel line-transect surveys
vessel line-transect surveys
rough sum of local abundances
vessel line-transect surveys
vessel line-transect surveys
vessel line-transect surveys
vessel line-transect surveys
vessel line-transect surveys; direct count of individuals
vessel line-transect surveys, group capture-recapture
vessel line-transect surveys
vessel line-transect surveys; sum of best group size estimates

vessel line-transect surveys
extrapolation of aerial line-transect surveys
aerial line-transect surveys
aerial line-transect surveys
aerial line-transect surveys

minimum N based on rough sum of available local estimates
sum of line-transect subpopulation estimates
estimate above plus previous estimates from other areas

vessel line-transect surveys
extrapolation of multiple aerial and vessel surveys

educated guess
vessel line-transect surveys
vessel line-transect surveys

vessel line-transect surveys
vessel line-transect surveys
extrapolation of IWC/IDCR estimates using Japanese sighting vessel data

sum of vessel line-transect surveys
vessel line-transect surveys
vessel line-transect surveys (low fin whale density season)
rough sumation of six estimates below
vessel and aerial line-transect surveys

vessel line-transect surveys
vessel line-transect surveys
vessel line-transect surveys
vessel line-transect surveys
vessel line-transect surveys
modeling CPUE and sightings data
extrapolation of IWC/IDCR estimates using Japanese sighting vessel data

census of photo-identified individuals
photo-identification recapture
genotype recapture
rough estimate

Method of N Estimation

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Reference

Table I.1. Abundance estimates reported for the 27 cetacean species and subspecies classified as vulnerable (VU), endangered (EN),
or
critically endangered (CR) by the IUCN Red List.
Appendix I. Abundance estimates reported for the 27 cetacean species and subspecies classified as vulnerable (VU), endangered (EN), or critically endangered (CR) by the IUCN Red List.

Review of abundance estimates for threatened cetaceans

Appendix I

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### Table I.1.

<table>
<thead>
<tr>
<th>Species or Subspecies</th>
<th>Status</th>
<th>Population or Area Estimated</th>
<th>Years(s)</th>
<th>CV</th>
<th>Method of N Estimation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eubalaena japonica</em></td>
<td>VU</td>
<td>Total distribution</td>
<td>2001</td>
<td>33</td>
<td>Rough estimate</td>
<td></td>
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<tr>
<td><em>Balaenoptera physalus</em></td>
<td>VU</td>
<td></td>
<td>1989</td>
<td>34</td>
<td></td>
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<tr>
<td><em>Phocoena phocoena relicta</em></td>
<td>VU</td>
<td></td>
<td>2002</td>
<td>34</td>
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<tr>
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<td>1996</td>
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</tbody>
</table>

### Notes:

- Taxonomic nomenclature is used according to the Society for Marine Mammalogy Taxonomy Committee [1]. In cases, nomenclature has changed since a referenced study was concluded, the abundance is reported under the currently accepted name for the taxa in the given geographic location.
- The most recent abundance is listed on the IUCN Red List unless a more recent, comprehensive published estimate was found using a Google scholar search for "scientific name" abundance and also using a taxon of former scientific name if recently changed. Not all estimates are representative of the entire range of the species or subspecies, and therefore do not represent the total abundance but rather a minimum estimate.
- The most recent "global" estimate is reported, unless more recent pairwise estimates exist to cover the majority of the distribution. In some cases, two estimates are listed if different methods were used for a similar time period.
- Known taxa occurred during the time of the estimate.
- The table retains the IUCN classification of critically endangered from its last evaluation in 2008, however its description specifies that it is "probably extinct" as the last documented sighting was in 2002. It is listed as extinct by the Society for Marine Mammalogy on Taxonomy [1].
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Appendix II

Low abundance and probable decline of the critically endangered Maui’s dolphin estimated by genotype capture-recapture

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Abstract

The New Zealand endemic Maui’s dolphin (*Cephalorhynchus hectori maui*) is considered ‘critically endangered’ by the IUCN as a result of decline due, in part, to fisheries-related mortalities. To estimate the abundance and trends of this subspecies, we used open-population capture-recapture models based on microsatellite genotyping of living and beachcast (dead) dolphins sampled between January 2001 and November 2007. A total of 82 genetic samples were available: 70 biopsy samples collected from living Maui’s dolphins and 12 necropsy samples collected from beachcast or floating carcasses, of which 5 showed evidence of fisheries entanglement. Microsatellite genotyping of up to 14 loci identified 54 individuals; 42 sampled alive on one or more occasions, one sampled alive, then found beachcast 2 years later, and 11 sampled only as carcasses, including 2 neonates. The sex ratio of the sample did not differ significantly from unity (25 males: 29 females). Using a POPAN model for live-capture records, the abundance of the super population available during the multi-year study was estimated to be $N = 87$ (95% CL, 59 to 158). Using a Pradel-like model modified to include both live-capture and beachcast records, the abundance of the population was estimated to be $N = 69$ (95% CL, 38 to 125) for the midpoint of the study in 2003. The results of both models suggested that the population was likely to be declining across the study period, although this trend could not be confirmed with 95% confidence. As the genotypes provide permanent marks of individual identity, continued genetic monitoring could provide improved confidence in the abundance and trends of this subspecies.
**Introduction**

The Hector’s dolphin, *Cephalorhynchus hectori*, is endemic to the coastal waters of New Zealand. The largest concentrations of Hector’s dolphins are found around the South Island (Slooten *et al.* 2004), where they form four discontinuous and genetically differentiated populations (Pichler *et al.* 1998, Pichler 2002, Hamner 2008). A small population also inhabits the west coast of the North Island, and is now recognized as a subspecies, the Maui’s dolphin, *C. hectori maui*, based on morphological differences and genetic isolation from the South Island populations (Pichler *et al.* 1998, Baker *et al.* 2002, Pichler 2002). The Maui’s subspecies is considered vulnerable to extinction (Dawson *et al.* 2001) and is classified as ‘critically endangered’ by the International Union for the Conservation of Nature (IUCN 2009) and ‘nationally critical’ under the New Zealand Threat Classification (Baker *et al.* 2010).

The Maui’s dolphin was once thought to be widely distributed along the west coast of the North Island, with occasional sightings along the east coast (Du Fresne 2010). Based on records of strandings and sightings (Dawson *et al.* 2001), this distribution has apparently contracted over the last 100 years. Since 1970, when systematic collection of stranding records began, the distribution of strandings has been concentrated along the northwestern coast from Dargaville in the north to Wanganui in the south (Figure II.1). Within this limited range, the distribution has contracted further over the last three decades (Dawson *et al.* 2001). Surveys of Maui’s dolphins since the late 1990s have reported the large majority of sightings along only 40 km of coastline from the Manakau Harbour to Port Waikato (Slooten *et al.* 2006, Du Fresne 2010). Estimates of abundance from small-boat and aerial surveys vary from 75 to 140 individuals, with relatively large confidence limits (Table 1; (Dawson and Slooten 1988, Martien *et al.* 1999, Ferreira and Roberts 2003, Slooten *et al.* 2006). Using extrapolated rates of fisheries mortality and estimated life history parameters from Hector’s dolphins, a population dynamic model suggests a substantial decline in abundance since the advent of nylon monofilament set nets in the late 1960’s (Martien *et al.* 1999).
Over the last decade, the New Zealand Ministry of Fisheries, with advice from the Department of Conservation, has implemented a series of fisheries closures along the northwest coast of the North Island in an effort to protect Maui’s dolphins. The first of these was a ban on commercial set nets within 4 NM and trawl nets within 1 NM of the coast from Maunganui Bluff (north of Dargaville) to Pariokariwa Point (north of New Plymouth; Figure II.1). This length of coast includes all sightings of Maui’s dolphins during systematic surveys from 2000 to 2009 (Du Fresne. 2010). Although announced in 2001, the ban was challenged in court by fisheries interests, and did not take effect until late 2002. In 2008, the Ministry extended the ban on commercial and recreational set nets from 4 NM to 7 NM offshore for this length of coastline and extended the trawl net ban to 4 NM for a section between Manukau Harbour and Port Waikato, considered to have the highest density of dolphins, and to 2 NM for the remaining area. Fisheries interests again lodged a legal challenge, requiring the Minister to reconsider this action (Ministry of Fisheries 2010). Following public submissions and review, the Minister reconfirmed his position and the ban went into force in 2010 (Minister of Fisheries 2010). Despite these protective measures and effort to improve estimates of abundance, there is no direct evidence for either a decline or increase in this population over the last decade.

Here we present results of the first minimum census and estimated abundance of Maui’s dolphins using microsatellite genotypes for individual identification. Genetic samples were collected from living individuals using a small biopsy dart (Krützen et al. 2002) and from beachcast carcasses during necropsy. Unlike previous sighting surveys of Maui’s dolphins, the biopsy-based genotyping allows for the accumulation of capture records over time, providing the potential to estimate trends in abundance through open-population models. Although now commonly used for estimating abundance of large whales (e.g., (Wade et al. 2010), relatively few studies have used genotype capture-recapture for dolphins (e.g., (Oremus et al. 2007). Genotype identification is particularly useful for Maui’s dolphins because living individuals show a low rate of natural markings for individual identification, e.g., only 10.5% of Hector’s dolphins are considered
identifiable (Gormley et al. 2005). Most natural marks also tend to diminish in beachcast
 carcasses, preventing the inclusion of these mortality events in capture-recapture models.

Methods

Sample collection
Small skin samples were collected from Maui’s dolphins during 20 small-boat surveys
along the west coast of the North Island, New Zealand using a minimally invasive biopsy
dart deployed from a modified veterinary capture rifle (Krützen et al. 2002). During each
survey, we attempted to travel a standard length of coastline (approximately 40 km) but
frequently departed from a longshore trackline depending on sighting of dolphins, as well
as sea conditions and weather. The surveys were undertaken during five spring/summer
sampling periods between January 2001 and December 2006 (see Supplementary
Material, Table II.1) and covered most of the known distribution of Maui’s dolphins, as
reported in recent aerial surveys (Du Fresne 2010). Given the very low density of
dolphins elsewhere, the majority of biopsy samples were collected in the current ‘core’
area of sightings from Manukau Harbour to Port Waikato (Figure II.1). Samples were
also collected by staff of the New Zealand Department of Conservation from all carcases
of beachcast or floating Maui’s dolphins reported between January 2001 and November
2007. Depending on the condition of the carcass, necropsies were conducted by staff
from the School of Veterinary Medicine, Massey University, and reported to the
Department of Conservation incident database (New Zealand Department of
Conservation 2010).

DNA extraction and genotyping
Samples were stored in 70% ethanol and archived at -80°C at the University of Auckland.
Genomic DNA was extracted from each skin sample (both biopsy and beachcast) by
digestion with proteinase K followed by a standard phenol-chloroform extraction
procedure (Sambrook et al. 1989). Sex was identified by co-amplification of X and Y
(sry) chromosome specific markers (Gilson et al. 1998). The sex ratio of individuals was compared to an expected 1:1 ratio using a two-tailed binomial distribution test (\(\alpha = 0.05\)). Approximately 400-700 bp of the mitochondrial (mt) DNA control region was sequenced for all samples using primers and methods described in (Oremus et al. 2007). Haplotypes were identified based on alignment to 360 bp reference sequences from all known Maui’s and Hector’s dolphin control region haplotypes (Pichler et al. 1998, Pichler and Baker 2000); using Sequencher v. 4.7 (Gene Codes Corporation).

Individual identification was inferred from genotypes of up to 14 microsatellite loci (Table II.2). Each locus was amplified individually and co-loaded with up to 5 other loci on an ABI 3730 for visualisation. The resulting peak profiles were automatically sized and visually verified using the computer programme GeneMapper®. Genotyping of a subset of samples were repeated independently by at least two of the authors (MV, DH or RH) and compared to assess and correct genotyping error. Multi-locus genotypes of the 82 samples were compared to identify matches using the program Cervus v. 3.0 (Kalinowski et al. 2007).

To avoid false exclusion due to even low levels of genotype error, initial comparison allowed for mismatching at up to 3 of the 14 loci (i.e., ‘relaxed matching’). Genotypes of the matches under the relaxed criteria were examined and repeated as necessary to correct genotyping error or to confirm true differences. Observed and expected heterozygosity and deviations from Hardy-Weinberg equilibrium were calculated in Arlequin v. 3.5.1.2 (Excoffier and Lischer 2010). Micro-Checker v. 2.2.3 (Oosterhout et al. 2004) was used to assess systematic genotyping error and the potential presence of null alleles, although true null alleles would not affect the individual identification. An initial estimate of genotyping error was calculated as a ratio of the number of allelic differences to the total number of alleles compared, based on the replicate amplification and sizing of a subset of samples (Bonin et al. 2004).
Capture-recapture models

The unique microsatellite genotypes were considered to represent a minimum census of the individual dolphins alive at some point during the study. The matching genotypes were considered to represent a set of recaptures of these unique individuals. Given the availability of samples from both living and beachcast individuals, we used two modelling approaches for capture-recapture: 1) POPAN models, as implemented in the program MARK (White and Burnham 1999), for estimating abundance from records of living dolphins, and 2) a bespoke modification of a Pradel model (Pradel 1996) formulated by one of the co-authors (JC), for estimating abundance and annual rates of change with the inclusion of records from the beachcast carcasses.

With POPAN, we estimated abundance and sex-specific abundance using the five annual sampling periods of live capture-recapture. We initially explored eight models representing all combinations of both constant and time-dependent specifications for capture probability ($p$), survival ($\phi$), and the probability of entry into the population ($\text{pent}$) (Arnason and Schwarz 1995). Preliminary model runs showed that the sparse recapture records could not support time-dependent estimates of $\phi$ or $\text{pent}$ (see Supplementary Material, Table II.2), reducing the number of acceptable models to two. The AICc as calculated in MARK was used in model selection. Goodness of fit (GOF) tests were used to evaluate assumptions of homogeneity of capture and survival, as well as transience (test 3.SR) and trap-dependence (test 2.CT), using the program U-CARE v. 2.02 (Choquet et al. 2009). As POPAN provides an estimate of the super population alive during the multi-year study period, we also reported annual estimates of abundance ($N$-hat) as an indication of trends and for comparison to the Pradel-like model. Log-normal confidence limits were calculated for all estimates of abundance.

The modified Pradel model allowed for multiple sampling events within years and for the inclusion of beachcast individuals, if mortality occurred subsequent to live sampling events (i.e., if the individual was available for capture prior to death). As with the POPAN analysis, the data could support a model with only a limited number parameters:
(i) the population size (referenced to January 2003); (ii) the annual mortality rate (as a fraction of population size); and (iii) the annual recruitment rate (the fraction of the population consisting of new recruits to the population). The model was fitted to the sequence of capture-recaptures by maximum likelihood on the assumption that each dolphin sampled is drawn randomly from the population alive at that time.

The three base parameters of the model are:
\( \rho \): instantaneous recruitment rate
\( \mu \): instantaneous mortality rate
\( N_0 \): population size in a fixed reference year

The derived parameters are:
\[
R = 1 - \exp(-\rho) \quad \text{annual fraction of new recruits in the population}
\]
\[
S = 1 - \exp(-\mu) \quad \text{annual survival rate as a fraction of the population}
\]
\[
r = \exp(\rho - \mu) - 1 \quad \text{annual rate of population change}
\]
\[
N_t = N_0 \exp((\rho - \mu)t) \quad \text{population size in year } t
\]

**Life history parameters**

Information on reproductive rates and assumed neonatal mortality was used to calculate a plausible range of recruitment rates for comparison to the model estimates. Following (Slooten 2007), we assumed the age at first reproduction to be 7-9 years and the interval between calving events to be 2-4 years. If we further assume a neonatal mortality rate of 0-20%, then these ranges can be converted to annual recruitment rates \( R \) by solving the balance equation:

\[
R = \frac{1}{2} p(1 - R)^t / T
\]

where:
- \( p \) neonatal survival rate
- \( t \) age at first calving
- \( T \) inter-calving interval (years)
The resulting range of values of $R$ can then be used to calculate the expected proportion of the population that is of reproductive age ($F$):

$$F = (1 - R)^t$$

**Results**

Between January 2001 and February 2006, a total of 70 biopsy samples were collected from living Maui’s dolphins during 20 small-boat surveys. During the same period, and extending into 2007, a total of 12 necropsy samples were collected from beachcast carcases (Table II.3; Supplementary Material, Table II.1). All living dolphins were judged to be adults based on observations at the time of the biopsy collection. The 12 beachcast specimens included two neonates: one found unaccompanied in November 2006 and one found in December 2006 with a mature female (also dead), assumed to be its mother (supported by shared alleles at each microsatellite locus). The remainder of the beachcast specimens were judged to be adults or juveniles based on length measurements (New Zealand Department of Conservation 2010). The majority of samples were collected between Manukau Harbour and Port Waikato (Figure II.1), consistent with the spatial distribution of sightings in recent aerial surveys (Du Fresne 2010).

The diversity of the microsatellite loci in Maui’s dolphins was low, with only 2-6 alleles each (Table II.2). The program MicroChecker showed no significant evidence of null alleles or other systematic genotyping errors for any of the loci. To estimate and correct genotyping error, genotyping at up to 14 loci was repeated for a subset of samples ($n=30$), including all potential matches identified using the ‘relaxed’ criterion. An initial error rate of 0.75% was calculated based on the comparison of 528 alleles from these replicates. All detected errors appeared to be the result of allelic dropout (i.e., homozygous in one run and heterozygous with one matching allele in the alternate run). These detected errors were corrected by reviewing the original genotype profiles or by repeating the amplification and genotyping of the locus. After correcting the detectable errors, the final error rate could not be measured but was, presumably, substantially lower than calculated initially (i.e., $<<0.75%$).
From the 82 tissue samples, the 14 microsatellite loci resolved 54 unique genotypes: 42 from biopsy samples of living dolphins, one from a biopsy sample of a living dolphin and from a carcass found beachcast two years later, and 11 from carcasses found beachcast only (Table II.3 and Supplementary Material, Table II.1). The overall probability of identity ($P_{(ID)}$) for the 14 loci was $6.4 \times 10^{-6}$ and the probability of identity for siblings ($P_{(ID)_{sib}}$) was $3.1 \times 10^{-3}$. Given the small number of samples and the presumed small size of the population, we considered the $P_{(ID)}$ sufficiently low to assume that the unique genotypes represented 54 individual dolphins alive at some time during the study. The 54 individuals showed a nearly equal sex ratio, with 25 males and 29 females (binomial test, $P = 0.68$). Mitochondrial DNA control region sequences (360 bp) showed no variation among any of the samples, supporting the previous report of a fixed haplotype (‘G’) unique to this subspecies (Pichler and Baker 2000).

After removal of replicate samples collected on the same day (considered pseudo-replicates, rather than recaptures) and the two beachcast neonates (considered unavailable for capture before death), there were 71 capture records involving 52 individuals: 40 individuals sampled once, 8 sampled twice, 1 sampled three times and 3 sampled four times (Supplementary Material, Table II.1). After grouping the live samples into five annual sampling periods, there were 59 capture records involving 43 individuals: 34 sampled once, 7 twice and 2 three times (Table II.3). All recaptures occurred within the primary sampling area south of the Manukau Harbour, providing no information on movement within the apparently limited distribution of this population. The three tests of goodness of fit in U-CARE showed no significant departure from homogeneity of capture, or significant evidence of transience or trap-dependence in the annual capture records ($P > 0.05$).

With POPAN, the best fitting model was $\phi_i(.) p(t) pent(.)$, followed by $\phi_i(t)p(t) pent(.)$ with a Delta AICc of 5. Following the rule of thumb for model selection with Delta AICc (Burnham and Anderson 2002), we considered $\phi_i(.) p(t) pent(.)$ the most appropriate for the data. This model makes the plausible assumption that capture probability varies by
year but survival and entry into the population were constant over the study period. Details of model selection and parameter estimates are included in Supplementary Material, Table II.2. The estimates of abundance for the ‘super population’ with this model was $N = 87$ (95% CL, 59 to 158). Annual estimates of abundance ($N$-hat) showed a decline from $N = 87$ in 2001, to $N = 59$ in 2006, although confidence limits were wide and overlapping for all years (Table II.4a). As expected from the sparse records, the estimate of survival was imprecise, $\phi = 0.93$ with 95% CL, 0.02 to 0.99. Probability of capture averaged 0.142, varying from a high of 0.243 in 2001 and 0.242 in 2003 to a low of 0.037 in 2002, the year with only 3 live capture records (Supplementary Material, Table II.2). Sex-specific estimates of abundance showed a better model fit with $\phi(t)p(t)\text{pent}(.)$ for females and $\phi(.p(.)\text{pent}(.))$ for males (see Supplementary Material, Table II.3). The estimate for females, $N = 37$ (95% CL, 27 to 68), was somewhat lower than for males $N = 58$ (95% CL, 32 to 143), but confidence limits were wide and overlapping.

With the Pradel-like model, the population was estimated to be $N = 69$ individuals (95% CL, 38 to 125) at the midpoint of the study in 2003. This was similar to the POPAN estimate of $N = 74$ (95% CL, 16 to 132) for the same year (Table II.4a). As with POPAN, the estimates of the other model parameters were imprecise (Table II.4b). The point estimate of 0.07 for the recruitment fraction is consistent with the range that would be expected based on life history parameters for Hector’s dolphins (0.058 - 0.111, see Table II.5). The annual survival rate was estimated to be 0.82, with 95% confidence limits of 0.40 to 0.97, and the annual rate of change was estimated to be -0.13 (e.g., a 13% decrease per year), with 95% confidence limits (-0.40 to +0.14) that spanned zero (e.g., we could not exclude a small probability that the population is increasing; Table II.4). Based on the life history analysis, the maximum feasible rate of annual population increase is about +0.11 (e.g., an 11% increase per year), assuming little or no juvenile mortality (Table II.5).
Discussion

Estimating the abundance and trends in populations of dolphins is challenging, especially if the population is small and its habitat is difficult to access. When sighting records are sparse, estimates of abundance will have wide confidence intervals and even a substantial trend in decline can go undetected because of lack of power in survey methods (Taylor et al. 2007). Maui’s dolphins offer a further challenge in lacking the distinctive natural markings required for photo-identification – a method widely used in estimating abundance and trends in populations of coastal dolphins (e.g., (Wilson et al. 1999). Given the limitations of individual identification, previous efforts to estimate the abundance of Maui’s dolphins have relied on distance sampling from small-boat and aerial surveys (Table II.1). These have established that the population is small but are unlikely to provide evidence of a trend in abundance, given the effort required for these surveys and the sparse sighting records. In the 2004 aerial surveys (Slooten et al. 2006), for example, nearly 2,000 km of effort recorded only eight sightings of Maui’s dolphins, from which abundance was estimated to be $N = 111$, with 95% CL of 48 to 252.

Our genotype-based individual identification represents an entirely independent estimate of abundance for Maui’s dolphins, allowing the accumulation of capture-recapture records over several years of sampling effort. Our estimates are not inconsistent with those from small-boat and aerial surveys (Table II.1), but suggest that the population is smaller than previously reported, probably less than 75 individuals in 2003. The capture-recapture analyses also suggest that the population was likely to be declining over the period of 2001 to 2006, perhaps at a rate of 13%/year. Together with previous studies, these results support the current IUCN classification of Maui’s dolphins as ‘critically endangered’ (IUCN 2009) and ‘nationally critical’ under the New Zealand Threat Classification (Baker et al. 2010). Along with the vaquita, Phocoena sinus (Jaramillo-Legorreta et al. 2007), the Maui’s dolphin is now likely to be one of the most endangered species or subspecies of cetaceans in the world.
Although it was not possible to confirm a decline in the abundance of Maui’s dolphins with 95% confidence, this apparent trend is consistent with the longer-term pattern of range contraction (Dawson et al. 2001) and the recorded mortality of 12 individuals during the study period. Of these 12 carcases, five showed signs of entanglement according to the Department of Conservation incident database (New Zealand Department of Conservation 2010; Table II.3). The five carcases showing signs of entanglement were all recovered in 2001 and 2002, before the set-net ban took effect in the study area (given the delay resulting from the legal challenge by the fisheries industry). The documented entanglement deaths of five individuals across the study period clearly exceed the ‘potential biological removal’ of one dolphin every 6.4 years, as calculated by (Slooten et al. 2006) using the formula for setting allowable human-related mortality under the US Marine Mammal Protection Act (Wade 1988). Although the other seven beachcast or floating carcasses found during our study period did not show evidence of fisheries-related entanglement, the stochastic nature of these non-anthropogenic mortality events and the potential for inbreeding effects in the small population place a high priority on eliminating any human-related mortality.

The apparent decline in abundance of Maui’s dolphins is also consistent with an apparent contraction in the range of this subspecies over the last few decades (Dawson et al. 2001). The majority of our biopsy samples were collected along only 40 km of coastline, from the mouth of the Manakau Harbour to Port Waikato (Figure II.1), despite efforts to survey the coastline to north and south of this ‘core area’. This limited distribution is consistent with the sightings during recent aerial surveys – six of the eight sightings in the 2004 surveys were located within the core area of our biopsy sampling (Slooten et al. 2006). The locations of beachcast carcases reported during our study were also consistent with our biopsy sampling and the aerial surveys – nine of the 12 carcases were found in, or immediately adjacent, to the core area (Figure II.1). The close agreement in spatial overlap from these three independent sources is strong evidence that the distribution of Maui’s dolphins is now extremely limited (IUCN 2009).
With longer-term capture-recapture records and increased sampling effort, it should be possible to establish a trend in abundance for Maui’s dolphins with greater statistical confidence. However, we do not consider that any management actions should be delayed in expectation of greater certainty in a decline, given the independent lines of evidence supporting this trend and the potential consequences of an ‘under-protection error’ (Taylor et al. 2007). The biopsy samples used here for individual identification also provided information on the sex ratio of this small population, which, at present, appears to be nearly equal. Additionally, the samples confirmed the genetic distinctiveness of the Maui’s dolphin subspecies, not only with respect to mitochondrial DNA, but also through population assignment tests based on the microsatellite genotypes (Hamner 2008). The ability to assign individuals through microsatellite genotypes is critical in supporting the subspecies classification of Maui’s dolphins and the testing the assumption of geographic closure of this population. Finally, our census of individual genotypes for the years of this study can serve as the baseline for the accumulation of lifetime recapture records and changes in effective population size in this critically endangered subspecies (Schwartz et al. 2007).

Acknowledgments

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Table II.1: Previous estimates of abundance (N), and associated 95% confidence limits (CL), for Maui’s dolphins, based on distance sampling from small-boat and aerial sighting surveys.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Applicable year(s)</th>
<th>N</th>
<th>Lower CL</th>
<th>Upper CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dawson and Slooten (1988)</td>
<td>1985</td>
<td>134</td>
<td>n.a.</td>
<td>n.a</td>
</tr>
<tr>
<td>Martien et al. (1999)</td>
<td>1985</td>
<td>140</td>
<td>46</td>
<td>280</td>
</tr>
<tr>
<td>Russell (1999)</td>
<td>1998</td>
<td>80</td>
<td>n.a.</td>
<td>n.a</td>
</tr>
<tr>
<td>Ferreira and Roberts (2003)</td>
<td>2001/02</td>
<td>75</td>
<td>48</td>
<td>130</td>
</tr>
<tr>
<td>Slooten et al. (2006)</td>
<td>2004</td>
<td>111</td>
<td>48</td>
<td>252</td>
</tr>
</tbody>
</table>

*aNote: The estimate and confidence intervals in Martien et al. (1999) were recalculated from the sightings reported in Dawson & Slooten (1988), i.e., these are not independently derived.

Table II.2: Microsatellite loci used for genotyping Maui’s dolphins. Observed (H_O) and expected (H_E) heterozygosity, deviations from Hardy-Weinberg equilibrium (P, unadjusted for multiple tests) were calculated for each locus based on the allele frequencies of the 54 individuals after removal of replicate samples.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Individuals</th>
<th># Alleles</th>
<th>H_O</th>
<th>H_E</th>
<th>P</th>
<th>P(ID)</th>
<th>P(ID)sib</th>
</tr>
</thead>
<tbody>
<tr>
<td>GT23*a</td>
<td>53</td>
<td>2</td>
<td>0.53</td>
<td>0.45</td>
<td>0.20</td>
<td>0.42</td>
<td>0.64</td>
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<td>GT509*a</td>
<td>26</td>
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<td>0.04</td>
<td>0.92</td>
<td>0.95</td>
<td>0.97</td>
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<tr>
<td>GT575*a</td>
<td>53</td>
<td>2</td>
<td>0.13</td>
<td>0.11</td>
<td>0.66</td>
<td>0.72</td>
<td>0.85</td>
</tr>
<tr>
<td>PPHO130*b</td>
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<td>2</td>
<td>0.15</td>
<td>0.17</td>
<td>0.40</td>
<td>0.69</td>
<td>0.83</td>
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<tr>
<td>PPHO137*b</td>
<td>47</td>
<td>2</td>
<td>0.47</td>
<td>0.48</td>
<td>0.85</td>
<td>0.40</td>
<td>0.62</td>
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<tr>
<td>PPHO142*b</td>
<td>52</td>
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<td>0.49</td>
<td>0.46</td>
<td>0.38</td>
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<td>TruGT51*c</td>
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<td>2</td>
<td>0.19</td>
<td>0.17</td>
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| Overall        | 54          | 2.8       | 0.41 | 0.40 | 6.4 x 10^-6 | 3.1 x 10^-3 |

Table II.3 (on next page): Annual capture-recapture records of 54 individual Maui’s dolphins based on genotypes from biopsy samples ($n = 70$) and beachcast specimens ($n = 12$; in black boxes). Replicates and within-year recaptures are shown separated by a comma. The beachcast specimens thought to have died as a result of fisheries entanglement are marked with an asterisk*. The two neonates are indicated with a pound sign #. The dates of the individual sampling events are provided in Supplementary Material, Table II.1.
Table II.3.

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Table II.4: Population estimates from genotype capture-recapture for Maui’s dolphins using a) POPAN with constant survival \((\phi)\), constant probability of entry \((pent)\) and time-variant capture probability \((p)\) for annual live capture records, and b) a modified Pradel model for multiple capture events, with live capture and beachcast records.

### a) POPAN model \(\phi(.)p(t)pent(.)\)

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<th>Upper 95% CL</th>
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<td>Annual abundance ((N-hat)), 2004</td>
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<td>33</td>
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<tr>
<td>Annual abundance ((N-hat)), 2006</td>
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<tr>
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<td>0.02</td>
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### b) Pradel-like model

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<th>Upper 95% CL</th>
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<td>Annual survival ((S))</td>
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Table II.5. Representative recruitment rates \((R)\) and the expected proportion mature individuals \((F)\) of Maui’s dolphins, assuming a range of values for inter-calving interval \((T)\), age of first calving \((t)\) and neonatal survival \((p)\) reported for Hector’s dolphins (Slooten 2007).

<table>
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<th>(t)</th>
<th>(p)</th>
<th>(R)</th>
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Figure II.1: Distribution of Maui’s dolphin samples collected between 2001 and 2007. The numbers of biopsy \((n = 70)\) and beachcast \((n = 12)\) samples are indicated in each of the areas delimited by the horizontal black lines. Black circles indicate the recovery locations of beachcast specimens, with numbers to indicate if multiple specimens were recovered in a similar location. The area shaded by diagonal lines indicates a ban on commercial and recreational set nets to 7 NM from shore and trawling out to 2 NM. The crosshatched area between the Manukau Harbour and Port Waikato indicates a ban on trawling out to 4 NM from shore.
Appendix III

Distribution, group characteristics and movements of the critically endangered Maui’s dolphin (Cephalorhynchus hectori maui)

Marc Oremus, Rebecca M. Hamner, Martin Stanley, Phillip Brown, C. Scott Baker, and Rochelle Constantine

Endangered Species Research
Inter-Research
Nordbunte 23 (+5, 28, 30),
21385 Oldendorf/Luhe, Germany
2012, 19: 1-10
Abstract

Maui’s dolphin *Cephalorhynchus hectori maui* is one of the most endangered subspecies of mammals, and yet its ecology is poorly known, partly because of the difficulty in following individuals throughout their range and over time. Here we combined group sightings (n = 45) from 23 boat surveys and genotype recaptures from biopsy samples (n = 73, 20 microsatellite loci) collected over 2 summers (2010 and 2011) to investigate along-shore distribution, group characteristics and individual movements of Maui’s dolphins. We found a clumped distribution extending along 139 km of coastline, with the highest density of dolphins between Manukau Harbour and south of Port Waikato, New Zealand. As recapture events (n = 32) revealed movements throughout most of their range, we suggest that the clumped distribution is driven by patchy food resources and/or social factors rather than by site fidelity. Mean group size was 4.7 (SD = 3.0), with several large aggregations (≥8 dolphins) containing a higher proportion of calves than smaller groups and thus probably acting as nursery groups. Group composition by sex was different in large and small groups, with more adult females in large groups. The majority of small groups contained individuals of both sexes, which contrasts with the pattern of sex segregation described in the South Island, New Zealand. A conservative estimate indicates that the mean along-shore range for Maui’s dolphins is at least 35.5 km, suggesting similarity to Hector’s dolphins. However, some aspects of Maui’s dolphin ecology as described here might have been affected by the recent history of population decline and low abundance.
Introduction

The Hector’s dolphin *Cephalorhynchus hectori* is endemic to the coastal waters of New Zealand, showing the most limited range of any marine cetacean other than the vaquita *Phocoena sinus* (Reeves *et al.* 2008). Two subspecies are currently recognized on the basis of morphological and genetic evidence (Pichler *et al.* 1998, Baker *et al.* 2002): the South Island population retains the common name of Hector’s dolphin *C. hectori hectori* and the North Island population is now referred to as Maui’s dolphin *C. hectori maui*. The conservation status of the Maui’s dolphin is of particular concern since its current population size is estimated at 55 individual (Hamner *et al.* 2012a). The population has been declining since the 1960s mainly because of substantial by-catch mortality in gillnet fisheries; today, it is thought to be only a fraction of its original size (Martien *et al.* 1999, Slooten *et al.* 2000, Baker *et al.* 2013). Consequently, the subspecies has been classified as Critically Endangered by the International Union for the Conservation of Nature (Reeves *et al.* 2008) and Nationally Critical under the New Zealand Threat Classification (Baker *et al.* 2010).

Maui’s dolphins are restricted in their range to the west coast of the North Island, but historical records of strandings and sightings suggest that their distribution has contracted over the last 100 yr (Russell 1999, Dawson *et al.* 2001). Recent systematic surveys and reported sightings indicate that their current distribution extends from Kaipara Harbour to Kawhia Harbour (a distance of approximately 190 km), with occasional sightings as far south as New Plymouth (Du Fresne 2010; Figure III.1). However, distribution of the subspecies is non-homogenous with the highest concentration within a 40 km stretch of coast, between Manukau Harbour and Port Waikato, in the centre of the subspecies’ range (Reeves *et al.* 2008, Du Fresne 2010). The dolphins are primarily observed within 2 km of the coastline (Ferreira and Roberts 2003, Slooten *et al.* 2005) but multiple sightings have confirmed that they also occur further offshore, extending out to the 7 NM (13 km) set-netting ban between Maunganui Bluff to Pariokariwa Point (Du Fresne 2010; Figure III.1).
The ecology of Hector’s dolphins in the South Island has been well studied in the last few decades (Dawson 2009), but in comparison, little is known about the group characteristics and individual movements of Maui’s dolphins. One reason for this is the relative absence of distinctive, long-term natural marks on the back and dorsal fin of this subspecies (the mark rate), which appears on only 10% of the individuals (M. Oremus, unpubl. data). This is similar to the mark-rate for Hector’s dolphins at Banks Peninsula (Gormley et al. 2005). Such a low mark rate, combined with a very small population size, limits the power of studies based on photo-identification. However, one way to overcome this problem is to use individual identification by DNA profiling or microsatellite genotyping, which provides a permanent and unique record for every individual. Skin samples as a source of DNA can now be collected from free-ranging small cetaceans using a lightweight biopsy dart fired from veterinary rifle with a variable pressure valve, ensuring minimum physical impact and behavioural response (Krützen et al. 2002, Noren and Mocklin 2012).

Systematic boat surveys were undertaken during February and March of two austral summers (2010 and 2011) on the west coast of New Zealand’s North Island. The primary goal of the surveys was to collect biopsy samples from Maui’s dolphins to provide new estimates of the current population size and level of genetic diversity (Hamner et al. 2012a). The combination of boat surveys and biopsy sampling provided an opportunity to investigate the distribution, group characteristics and movements of this Critically Endangered subspecies.

Materials and Methods

Surveys and data collection
Systematic coastal boat surveys were undertaken on a 7 m rigid-hulled boat with twin 90 hp outboard engines during two consecutive austral summer periods from 4 February to 2 March 2010 (n = 12) and 14 February – 10 March 2011 (n = 11). All surveys were
conducted along the west coast of the North Island of New Zealand. In order to maximize
the number of group encounters, effort was mostly (94%) concentrated within 2 km from
shore, where the summer concentration of Maui’s dolphins is higher (Slooten et al.
2005). Surveys were initiated only in Beaufort sea-states ≤ 2. On occasion, the sea-state
reached Beaufort 3 during the survey and on almost all occasions occurred during the
inbound journey. In these situations we considered whether the conditions still allowed
marine mammal sightings and continued effort on all occasions.

The surveys covered most of the current alongshore known range of Maui’s dolphins,
from North Kaipara to New Plymouth (Figure III.1). Four observers scanned 360°
around the vessel using the naked eye whilst travelling at ~15 to 20 knots. Once a group
was sighted, the boat was immediately slowed down to less than 5 knots in order to
approach the dolphins. A group is defined here as a spatial aggregation of dolphins that
appear to be involved in a similar activity (Shane et al. 1986). For each group encounter,
location using a global positioning system (GPS) was recorded, and group size was
estimated visually using minimum and maximum estimate when exact size was uncertain.
In such cases, the best estimate was calculated as the average between minimum and
maximum size. The number of calves (i.e., assumed to be less than one year old and less
than one meter long; Webster et al. 2010) in the group were noted if present.

The group encounter was ended when dolphins could not be approach any more at slow
speed or when we visually estimated that we had biopsied (see ‘Molecular methods’ for
details) the majority of individuals over 1 yr of age in the group and therefore the risk of
biopsying the same individual again was considered extremely likely. Observers made
sure that the dolphins were left behind when continuing the surveys, and if necessary, the
boat was sped up to avoid being followed by some individuals. Group encounters were
considered independent even though there was a possibility that individuals may be
encountered more than once per survey as a consequence of changes in group
composition over short periods of time. Even though dolphins were left behind before
continuing with the survey, individuals could leave the group and join with other
dolphins, thereby forming another group. However, this situation represented only a
small proportion of our re-sightings of individuals dolphins. Following Rayment et al.
(2009), we summarized the spatial distribution of survey effort by dividing the coastline
into sections of 5 nautical miles (9.3 km) in length and counting the number of times that
each section was visited (Figure III.1). To avoid pseudo-replication, each section was
counted once per day.

To investigate the relative along-shore density of Maui’s dolphins, we calculated the
number of groups and individuals encountered per visit in each of the sections, as
described above. To minimise the likelihood of encountering groups more than once,
only data recorded from the outbound journeys were used in the analyses of density and
group characteristics.

**Molecular methods**

The Paxarms system© and lightweight biopsy darts (about 21.5 g) were employed to
collect biopsy samples (Krützen et al. 2002). Darts are equipped with a stainless steel
biopsy head (5 mm diameter x 9 mm length) sterilised to minimise the risk of wound
infection. This is, to our knowledge, the smallest cutting head available to collect
cetacean biopsies. Efforts were made to minimise sampling individuals more than once,
but only few individuals had dorsal fin nicks which would have allowed individual
identification of dolphins that had been sampled. Therefore, efforts were made to identify
individuals with fresh biopsy marks; however, this was not possible if dolphins only
presented the side of their body that had not been biopsied to the observers. In addition,
the very small size of the marks inflicted by the biopsies and the fast movements of
Maui’s dolphins meant that it was not always possible to observe prior biopsy marks.
Dolphin response to all sampling events was recorded to ascertain the level of responses
(Noren and Mocklin 2012; see Table S1 in the supplement at www.int-res. com/articles
Skin samples were stored in 70% ethanol at -20°C. To determine the sex and identity of individual dolphins, genomic DNA was extracted from each skin sample by digestion with proteinase K followed by a standard phenol-chloroform extraction procedure (Sambrook et al. 1989), as modified for small samples (Baker et al. 1994). Sex was identified by co-amplification of X and Y (sry) chromosome specific markers (Gilson et al. 1998).

A total of 20 microsatellite loci previously developed for cetaceans were amplified and genotyped using an ABI3730. We used: 415/416 (Amos et al. 1993), EV14, EV37, EV94 (Valsecchi and Amos 1996), GT23, GT211, GT575 (Bérubé et al. 2000), KWM9b (Hoelzel et al. 2002), KWM12a (Hoelzel et al. 1998), MK5, MK6 (Krützen et al. 2001), PPHO110, PPHO130, PPHO142 (Rosel et al. 1999), SGUI06, SGUI07, SGUI16, and SGUI17 (Cunha and Watts 2007), TexVet5 (Rooney et al. 1999), TtruGT48 (Caldwell et al. 2002). Amplification reactions for ‘SGUI’ loci followed Cunha & Watts (2007) and all others were set up as 10mL reactions containing 1x PCR II buffer, 1.5mM MgCl2, 0.4mM each primer, 0.2mM dNTP, 0.125U Platinum Taq (Invitrogen) and 10-20ng/mL DNA template and amplified using the following thermocycling profile: 93°C 2 min; (92°C 30s, Tₐ 45s, 72°C 50s) x 15; (89°C 30s, Tₐ 45s, 72°C 50s) x 20; 72°C 3 min (where Tₐ is the annealing temperature, see Hamner et al. (2012a) for details). GENEMAPPER v. 3.7 (Applied Biosystems) was used to bin and visually verify the resulting peaks. Each amplification and sizing run included a negative control to detect contamination and a set of seven internal control samples to standardise allele binning with previous genotyping runs and estimate genotyping error, as recommended by Bonin et al. (2004).

Probability of identity ($P_{ID}$) and probability of identity of siblings ($P_{IDsibs}$) were calculated using the program GENALEX 6.1 (Peakall and Smouse 2005). Microsatellite genotypes were then compared for the purposes of individual identification, both within and across sampling years, using the program CERVUS 3.0.3 (Kalinowski et al. 2007). Initial comparisons allowed for mismatching of up to five loci (‘relaxed matching’) to prevent
false exclusion due to genotyping error. Relaxed matches were visually examined for potential allelic dropout, as well as matching sex and mtDNA haplotype, and repeated to confirm or correct the genotype as necessary.

Results

Effort and distribution

Survey effort was evenly spread between the two field seasons (both covering a ~ 3 week period during February – March each year) with a total of 178 h spent on the water and a total distance covered of 4010 km (Table III.1). Most effort was concentrated within 2 km of shore, but we occasionally surveyed beyond this limit during the inbound journey (between 2 and 5 km from shore), representing about 6% of search effort. A total of 63 groups were encountered. Among these, 18 encounters occurred during the inbound journey. Because of the lack of distinctive marks, it was not possible to determine if these groups had already been seen on the outbound journey, and therefore, they were excluded from the analyses of density and group characteristics to avoid replication. This resulted in a minimum of 45 independent groups with an arithmetic mean (hereafter referred as “mean”) of 2 groups encountered per day (range = 0 to 6 groups/day). There was no significant difference in the rate of group encounters between the two years (Table III.1). Dolphins were observed on 87 % \((n = 20)\) of surveys, with only three unsuccessful surveys covering the northern (Kaipara Harbour to Bayly’s Beach) and southern (Raglan Harbour to New Plymouth) limits of the survey area (Figure III.1). There were no sightings in any of the partially surveyed harbours, including Manukau, Raglan and Kaipara Harbours.

The northernmost sighting was just south of the Kaipara Harbour (36° 28’ S) while the southernmost sighting was north of Raglan (37° 38’ S) (Figure III.2). Overall, the alongshore distribution was similar between the two surveys (Figure III.2), but in both years we found two areas with higher densities of sightings indicating a non-random
distribution. The first area of high-density was observed south of the Kaipara Harbour between sections 8 and 12 (36° 26’ S to 36° 47’ S), where a mean of 0.5 groups and 1.2 dolphins were encountered per section (Figure III.1). A second area of high density extended from south Manukau Harbour to north of Raglan, between sections 17 and 24 (37° 07’ S to 37° 37’ S, Figure III.1). In this region, the group encounter rate was fairly similar to that of the south Kaipara Harbour region with 0.6 groups per section, but the mean number of individuals was much higher with 3.3 dolphins encountered per section. Furthermore, within this second area, the distribution of group and individual density was found to be slightly bimodal with a first peak centred on Hamilton’s Gap and a second one south of the Waikato River mouth (Figure III.1).

**Biopsy collection and processing**

A total of 73 skin samples were collected (2010, \( n = 37 \); 2011, \( n = 36 \)). Each sample was genotyped for an average of 19 loci and a low error rate of 0.4% was estimated initially, after which genotypes were repeated for confirmation or correction as necessary. We calculated an overall \( P_{ID} \) of \( 7.3 \times 10^{-8} \) and a \( P_{IDsib} \) of \( 4.0 \times 10^{-4} \). Given this low probability of a match by chance and the small size of the population, we assumed that samples with matching genotypes were replicates (i.e., genotype re-captures) of the same individual. Comparison of genotypes revealed that 32 biopsies were re-sampling events, giving a final total of 41 individual dolphins.

Genetic sex identification from the biopsy samples revealed that 45 samples were from females and 28 were from males. Although the difference is substantial, this ratio does not significantly deviate from a theoretical 1:1 ratio (exact binomial test of goodness of fit, \( P = 0.06 \)). After excluding replicates, the sex ratio was 25 females and 16 males, which also was not significantly different from expectation (\( P = 0.211 \)).
**Group characteristics**

Mean group size over the two years was estimated to be 4.7 individuals (SD = 3.0, median = 4) based on best visual count estimates, ranging from 1 to 14 per group. Mean size was slightly larger in 2010 than 2011 (Table III.1), but the difference was not significant (Kruskal-Wallis test, \(H = 0.664, p = 0.415\)). Large aggregations (defined here as \(\geq 8\) dolphins, which represent the tail of the group size distribution, Figure III.3) were frequently observed, representing 25% of the groups in 2010 (\(n = 6\)) but only 14% in 2011 (\(n = 3\)) (Figure III.2). Most of these large aggregations were found in the high-density area south of Manukau Harbour (\(n = 7\)), but they were also observed near Waikato River mouth (\(n = 1\)) and north of Raglan Harbour (\(n = 1\)). A larger proportion of solitary dolphins were observed in 2011 (19%) compared to 2010 (8%) (Figure III.3).

Calves were found in 23% (\(n = 9\)) of groups. The mean size of groups containing calves was 8.2 (SD = 3.5), which was significantly larger than groups without calves (\(H = 11.905, p < 0.001\)), even when calves were excluded from the analysis (Kruskal-Wallis test, \(H = 5.923, p < 0.05\)). A total of 11 sightings of calves were recorded across the two surveys with a maximum of two calves observed within the same group. Ten of these sightings were made in 2010 within eight different groups while only one sighting of one calf was made in 2011. It was not possible to determine how many unique calves were present among the 10 sightings made in 2010, but we can provide a minimum estimate of three calves in the population during that summer by combining sightings of groups encountered on a single outbound journey.

We used molecular sexing from biopsies to compare sex composition in large groups (\(\geq 8\) individuals) versus small groups. We collected a total of 15 samples from females and 5 from males in large groups, while 10 were from females and 7 from males in small groups. Despite a noticeable difference in these proportions, an exact binomial test of goodness of fit revealed that sex capture within large and small groups is not significantly
different from null expectation ($P = 0.051$). Since this result could be explained by small sample sizes, we also looked at sex composition with the inclusion of all 63 groups encountered during the study ($i.e.$, including groups and samples from return trips). Doing so, we found that, in large groups, 23 samples were from females and 8 from males (ratio 2.8:1), while, in small groups, 24 were from females and 18 were from males (ratio 1.3:1). In this case, exact binomial test indicates significant difference ($P < 0.05$) in sex composition according to group size.

Partial information on group sex composition could be obtained from 13 unique groups from which we sampled more than one individual (mean = 2.4, range = 2 to 5). Overall, we identified males and females together within eight groups (62 %). According to group size, we found that 67% of large groups and 57% of small groups were composed of individuals from both sexes.

**Movements of Individuals**

Individual movements were documented by examining the sampling locations of replicate samples from the same individual. On nine occasions, re-sampling happened within the same day (Figure III.2, Table IV.5). Distances between same day re-samples ranged from 0.32 km within 13 minutes to 11.33 km within 2.5 hours (Table IV.5). Distances between recaptures within years but on different days ($n = 11$) ranged from 0.91 km for an individual re-sampled two days later to 78.62 km for an individual re-sampled 19 days later (Figure III.2, Table IV.5). There was no correlation between distance and number of days between re-sampling events ($R^2 = 0.158$, $p = 0.226$). Dolphins showed no clear direction of movement, with individuals moving both north and south. There was no significant difference in the within-year distance travelled for males and females (Kruskal-Wallis test, $H = 1.5$, $p = 0.221$).
Longer-term individual movements inferred from re-samples between years ($n = 12$) were of a similar scale to the short-term within-year movements, ranging from 0.88 km over 372 days to 80.43 km over 375 days (Figure III.2, Table IV.5). There was no significant difference in the mean distances between within-year re-sampling (excluding within-day re-sampling) and between-year re-sampling (Kruskal-Wallis test, $H = 0.124$, $p = 0.725$). The individual sampled across the greatest distance was a female who travelled from south of Kaipara Harbour to south of Manukau Harbour. Interestingly, in 2011, she travelled back to south of Kaipara Harbour within 19 days. There was no significant difference in the between-year distance travelled for males and females ($H = 0.321$, $p = 0.571$).

A total of six dolphins ($n = 3$ females, $n = 3$ males) were genetically recaptured on three ($n = 5$) or four ($n = 1$) days. These were used to assess the minimum along-shore home range for Maui’s dolphins. The mean distance between the two most extreme locations for these individuals was 35.50 km (SE = 4.03).

**Discussion**

The distribution pattern of Maui’s dolphins was found to be very similar over the two summers of our study (Figures III.1 and III.2); it indicates that their current core area of near-shore distribution extends along a 139 km stretch of coast from Kaipara Harbour to north of Raglan Harbour in the south, at least during the summer months. This distribution is largely in agreement with Du Fresne (2010), who synthesized results from surveys on the distribution of Maui’s dolphins conducted between 2000 and 2009. However, Du Fresne (2010) indicated that Maui’s were regularly observed as far south as Kawhia Harbour, whilst in our study the southernmost sightings were north of Raglan Harbour despite reasonable search effort further south in primarily good weather conditions ($\leq$ Beaufort 2) (Figure III.1). Although we recognised that opportunistic sightings of Maui’s dolphins are still occurring just south of Raglan Harbour, our results
suggest that the current core area of near-shore distribution of Maui’s dolphins might be smaller than previously assessed. It must be noted that sporadic sightings and by-catch events of Maui’s or Hector’s dolphins are still occurring in the Taranaki region, but the subspecies status for these is largely unresolved. Nevertheless, there is a need to monitor the status of the species in this area. Further contraction of the core distribution range is not unexpected considering that their population size might still be declining (Baker et al. 2013). The hypothesis that a low number of resident individuals occur only at the limits of the sub-species range (Du Fresne 2010) seems less likely now that we have shown that Maui’s dolphins commonly travel long distances within their current range. Therefore, the dolphins found along the coastline between Taranaki and Wellington will prove to be extremely interesting in the future, and we suggest surveys of this area be a priority.

The highest concentration of dolphins within the core area was found between Manukau Harbour and south of Port Waikato, confirming previous findings (Reeves et al. 2008). Our estimates of group and individual density were corrected by the number of visits to each section of the range, so this pattern is not due to the search effort being more intensive in this area, a concern that was expressed in previous surveys (Du Fresne 2010). The absence of sightings in the surveyed harbours, and in particular the Manukau Harbour, supports other findings that these habitats are used only occasionally by Maui’s dolphins (Rayment et al. 2011).

The sightings made during our surveys suggest a clumped distribution within the sub-species’ core area (Figure III.1). Indeed, a gap in distribution was observed between Muriwai Beach and Manukau Harbour despite covering this area multiple times over the two surveyed periods (Figure III.1). This result was surprising since previous studies conducted during summer time typically found a substantial number of groups within this area (Ferreira and Roberts 2003, Slooten et al. 2005). The reason for the absence of sightings during our surveys is unknown. Maui’s dolphins are at least crossing the zone from time to time since multiple movements were documented between south Manukau
and the northern part of the range. However, a shift in distribution related to further decline in abundance in recent years is an alternate explanation for this pattern, and therefore, further systematic surveys should be conducted in this area.

Further clumping in distribution was evident within the core area, with a peak south of Manukau Harbour, roughly in front of Hamilton’s Gap, and a peak south of Waikato River Mouth (Figure III.1). This pattern was not described in previous studies, although sightings from these surveys seem to indicate a similar trend (Du Fresnne 2010). Overall, the clumped distribution could be a reflection of site fidelity in Maui’s dolphins with some areas being more densely inhabited than others. However, the analysis of movements using genetic capture-recapture events shows multiple exchanges between the high and low density areas and thus, poor support for site fidelity. In fact, long-distance movements by males and females supports the findings that there is no population genetic structure within Maui’s dolphins’ current range (Hamner et al. 2012b). Instead of population substructure, we suggest that the distribution of Maui’s dolphins along the coast is most likely driven by uneven density of food resources and/or social factors such as mating behaviour or groups joining to reduce the risk of predation.

Assessment of the minimum along-shore home-range for Maui’s dolphins (35.50 km) was marginally larger than the mean home-range estimated for Hector’s dolphins at Banks Peninsula (33.01 km, Rayment et al. 2009), which suggests no major difference between the two subspecies. However, our estimate is conservative since it is based on three or four sightings per individual only compared to 10 or more sightings used by Rayment et al. (2009) to estimate Hector’s dolphin range at Banks Peninsula. The size of an individual’s or population’s home range is often influenced by prey distribution and productivity, with species in areas of high productivity showing smaller home ranges than species in areas of low productivity (Harestad and Bunnel 1979). However, demographic changes may be another factor influencing the home range of individuals from a depleted population such as Maui’s dolphins. It has been shown in other species
that a lower density of potential breeding mates could result in increased home ranges of individuals in search of mating opportunities (Loveridge et al. 2009).

Our investigation of group characteristics also gave some interesting insights into Maui’s dolphin ecology. We found that groups with calves were significantly larger than groups without calves. This pattern points to the formation of nursery groups where adults associate to form larger aggregations than usual in the presence of calves. Large groups were also found to have a sex-ratio bias toward adult females. This supports results from Banks Peninsula where all adult Hector’s dolphins associating with mothers and calves appear to be females (Webster et al. 2009). However, some males were also biopsied within large groups of Maui’s dolphins (although in smaller proportions than females), indicating that, within this subspecies, adults in the presence of calves are not exclusively female. The number of calf sightings was much more prevalent in 2010 \((n = 10)\) than in 2011 \((n = 1)\), and although it was not possible to determine the exact number of unique calves encountered in 2010, a minimum estimate of three calves suggests that there was a difference between the two research periods. This difference is unlikely to be due to a seasonal effect since both surveys were conducted almost at the same time of year, though we cannot fully discount this possibility. Female Hector’s dolphin are known to have a 2 to 3 year calving interval (Slooten and Lad 1991), which is likely to be similar in Maui’s dolphins. Therefore, alternative explanations could be a difference in yearly calving rates with synchronized calving or differences in calf mortality between years. Given the status of Maui’s dolphins this question should be investigated further. Note that synchronized calving could be favoured as a strategy against predator pressure, especially in a depleted population where females form nursery groups and do not reproduce every year.

Webster et al. (2009) suggested that Hector’s dolphin groups are highly segregated by sex, in particular small groups \((\leq 5 \text{ individuals})\) were found to contain both male and female adults only 9% of the time. Here, evidence from biopsy sampling show that at
least 57% of small groups contained both males and females, and this result is conservative as we only partially sampled the groups (36% of individuals sampled per group with at least one biopsy). This minimum estimate suggests that composition of small groups in Maui’s dolphins differs from the pattern observed in Hector’s dolphins at Banks Peninsula. Difference in sex composition within groups could reflect a different pattern of social organisation for both subspecies.

The mean group size during our study was substantially larger than previous estimates for Maui’s dolphins derived from aerial surveys (Table III.2). The reason(s) for this discrepancy is most likely due to differences in sampling methods. The fact that our boat surveys were strictly along-shore, in contrast to most aerial surveys, could reflect a tendency toward larger groups near the coastline. A preference for coastal and shallow waters has been shown for nursery groups in several species of delphinids (e.g., Mann et al. 2000), and we showed here that presumed nursery groups in Maui’s dolphins are significantly larger. This behaviour could be driven by several factors such as predator avoidance, foraging strategy, or mating behaviours (Connor 2000). Secondary explanations for discrepancy in mean group size estimates are possible. Indeed, aerial surveys might be more prone to under-estimating group sizes by missing individuals, especially for Maui’s dolphins which reach a maximum length of about 1.5 m and usually inhabit turbid waters (Dawson 2009). Conversely, boat attraction could bias group size estimates up when using boat-based surveys.

In conclusion, we have shown that, at least for the austral summer months of February and March, Maui’s dolphins have a smaller frequently used core area than previously reported. This is of concern as there are only two main areas of high-density sightings throughout their range. Despite these clumped areas of high density, Maui’s dolphins were found to travel long distances over the subspecies’ range. Therefore, it is absolutely necessary to maintain and maybe increase protection from anthropogenic threats throughout their entire range. Our results further suggest that several aspects of Maui’s
dolphins’ ecology could be influenced by the precarious status of the population, strengthening the need for further monitoring and investigation of their population dynamics and social organisation.

Acknowledgments

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References


Table III.1: Summary of survey effort and Maui’s dolphin group encounters on outbound surveys. Surveys were conducted from 4 February–2 March 2010 and 14 February–10 March 2011.

<table>
<thead>
<tr>
<th></th>
<th>2010</th>
<th>2011</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of surveys</td>
<td>12</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>Hours on effort</td>
<td>97</td>
<td>81</td>
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</tr>
<tr>
<td>Distance travelled (km)</td>
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<td>1893</td>
<td>4010</td>
</tr>
<tr>
<td>Number of groups</td>
<td>24</td>
<td>21</td>
<td>45</td>
</tr>
<tr>
<td>Mean number groups/day</td>
<td>2.0</td>
<td>1.9</td>
<td>2.0</td>
</tr>
<tr>
<td>Mean group size (SD)</td>
<td>5.1 (3.4)</td>
<td>4.1 (2.5)</td>
<td>4.7 (3.0)</td>
</tr>
</tbody>
</table>

Table III.2: Summary of mean group size estimates (with SD in parentheses) from previous research on Maui’s dolphins. Coastal habitat is defined here as the stretch of water extending from the coastline to 9.3 km offshore. Offshore habitat is defined as the area between the coastline and up to 9.3 km offshore.

<table>
<thead>
<tr>
<th>Source</th>
<th>Survey years</th>
<th>Season</th>
<th>Survey type</th>
<th>Habitat</th>
<th>Mean group size (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferreira and Roberts (2003)</td>
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<td>Dec-Mar</td>
<td>aerial</td>
<td>Coastal</td>
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<td>Slooten et al. (2006)</td>
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<td>aerial</td>
<td>Coastal Offshore</td>
<td>1.43</td>
</tr>
<tr>
<td>Rayment &amp; DuFresne (2007)</td>
<td>2007</td>
<td>Oct</td>
<td>aerial</td>
<td>Coastal Offshore</td>
<td>1.31 (0.48)</td>
</tr>
<tr>
<td>Childerhouse et al. (2008)</td>
<td>2008</td>
<td>May</td>
<td>aerial</td>
<td>Coastal Offshore</td>
<td>1.2 (0.4)</td>
</tr>
<tr>
<td>Webster &amp; Edwards (2008)</td>
<td>2008</td>
<td>Mar</td>
<td>aerial</td>
<td>Coastal</td>
<td>1.83 (1.27)</td>
</tr>
<tr>
<td>Stanley (2009)</td>
<td>2009</td>
<td>Jun-Jul</td>
<td>aerial</td>
<td>Coastal Offshore</td>
<td>1</td>
</tr>
<tr>
<td>Present study</td>
<td>2010/11</td>
<td>Feb-Mar</td>
<td>boat</td>
<td>Coastal</td>
<td>4.7 (3.0)</td>
</tr>
</tbody>
</table>
Maui’s dolphins are restricted in their range to the west coast of the North Island, but historical records of strandings and sightings suggest that their distribution has contracted over the last 100 yr (Russell 1999, Dawson et al. 2001). Recent systematic surveys and reported sightings indicate that their current distribution extends from Kaipara Harbour to Kawhia Harbour (a distance of approximately 190 km), with occasional sightings as far south as New Plymouth (Du Fresne 2010; Fig. 1). However, distribution of the subspecies is non-homogenous, with the highest concentration within a 40 km stretch of coast, between Manukau Harbour and Port Waikato, in the centre of the subspecies’ range (Reeves et al. 2008, Du Fresne 2010). The dolphins are primarily observed within 2 km of the coastline (Ferreira & Roberts 2003, Slooten et al. 2005), but multiple sightings have confirmed that they also occur further offshore, extending out to the 7 n mile (13 km) set-netting ban between Maunganui Bluff to Pariokariwa Point (Du Fresne 2010, Fig. 1).

The ecology of Hector’s dolphins in the South Island has been well studied in the last few decades (Dawson 2009), but in comparison, little is known about the group characteristics and individual movements of Maui’s dolphins. One reason for this is the relative absence of distinctive, long-term natural marks on the back and dorsal fin of this subspecies (the mark rate), which appears on only 10% of the individuals (M. Oremus unpubl. data). This is similar to the mark rate for Hector’s dolphins at Banks Point (2005).
Figure III.2. *Cephalorhynchus hectori maui*. Distribution of Maui’s dolphin group encounters in (A) 2010 and (B) 2011, including (C) group size information and individual movements within day, within year and between years as detected by genotype recaptures.
Group characteristics

Mean group size over the 2 years was estimated to be 4.7 individuals (SD = 3.0, median = 4) based on best visual count estimates, ranging from 1 to 14 per group. Mean size was slightly larger in 2010 than 2011 (Table 1), but the difference was not significant (Kruskal-Wallis test, $H = 0.664, p = 0.415$). Large aggregations (defined here as $\geq 8$ dolphins, which represent the tail of the group size distribution; Fig. 3) were frequently observed, representing 25% of the groups in 2010 ($n = 6$) but only 14% in 2011 ($n = 3$) (Fig. 2).

Most of these large aggregations were found in the high density area south of Manukau Harbour ($n = 7$), but they were also observed near the Waikato River mouth ($n = 1$) and north of Raglan Harbour ($n = 1$). A larger proportion of solitary dolphins were observed in 2011 (19%) compared to 2010 (8%) (Fig. 3).

Calves were found in 23% ($n = 9$) of groups. The mean size of groups containing calves was 8.2 (SD = 3.5), which was significantly larger than groups without calves ($H = 11.905, p < 0.001$), even when calves were excluded from the analysis (Kruskal-Wallis test, $H = 5.923, p < 0.05$). A total of 11 sightings of calves were recorded across the 2 surveys, with a maximum of 2 calves observed within the same group. Ten of these sightings were made in 2010 within 8 different groups, while only 1 sighting of 1 calf was made in 2011. It was not possible to determine how many unique calves were present among the 10 sightings made in 2010, but we can provide a minimum estimate of 3 calves in the population during that summer by combining sightings of groups encountered on a single outbound journey.

We used molecular sexing from biopsies to compare sex composition in large groups ($\geq 8$ ind.) versus small groups. We collected a total of 15 samples from females and 5 from males in large groups, while 10 were from females and 7 from males in small groups. Despite a noticeable difference in these proportions, an exact binomial test of goodness of fit revealed that sex capture within large and small groups is not significantly different from null expectation ($p = 0.051$). Since this result could be explained by small sample sizes, we also looked at sex composition with the inclusion of all 63 groups encountered during the study (i.e. including groups and samples from return trips). Doing so, we found that, in large groups, 23 samples were from females and 8 from males (ratio 2.8:1), while in small groups, 24 were from females and 18 were from males (ratio 1.3:1). In this case, exact binomial test indicates significant difference ($p < 0.05$) in sex composition according to group size.

Partial information on group sex composition was obtained from 13 unique groups from which we sampled more than 1 individual (mean = 2.4, range = 2 to 5). Overall, we identified males and females together within 8 groups (62%). According to group size, we found that 67% of large groups and 57% of small groups were composed of individuals from both sexes.

Movements of individuals

Individual movements were documented by examining the sampling locations of replicate samples from the same individual. On 9 occasions, re-sampling happened within the same day (Fig. 2, see Table S2 in the supplement at www.int-res.com/articles/supp.pdf).

Distances between same day re-samples ranged from 0.32 km within 13 min to 11.33 km within 2.5 h (Table S2). Distances between recaptures within years but on different days ($n = 11$) ranged from 0.91 km for an individual re-sampled 2 d later to 78.62 km for an individual re-sampled 19 d later (Fig. 2, Table S2). There was no correlation between distance and number of days between re-sampling events ($R^2 = 0.158, p = 0.226$). Dolphins showed no clear direction of movement, with individuals moving both north and south. There was no significant difference in the within-year distance travelled for males and females (Kruskal-Wallis test, $H = 1.5, p = 0.221$).

Longer-term individual movements inferred from re-samples between years ($n = 12$) were of a similar scale to the short-term within-year movements, ranging from 0.88 km over 372 d to 80.43 km over 375 d (Fig. 2, Table S2). There was no significant difference in the mean distances between within-year re-samples.

Figure III.3. *Cephalorhynchus hectori maui*. Group sizes of Maui’s dolphins encountered in 2010 and 2011 (outbound journeys only).
Appendix VI

Estimating the abundance and effective population size of Maui’s dolphins using microsatellite genotypes from 2010–11, with retrospective matching to 2001–07

Rebecca M. Hamner, Marc Oremus, Martin Stanley, Phillip Brown, Rochelle Constantine and C. Scott Baker
Summary

Estimating and monitoring trends in abundance and effective population size are key factors for planning and evaluating actions to conserve the critically endangered Maui’s dolphin (*Cephalorhynchus hectori maui*). Our work continues genetic monitoring of the Maui’s dolphin subspecies by using DNA profiles to estimate the current abundance and effective population size, as well as to document movements of individuals.

Small-boat surveys dedicated to the collection of dart-biopsy samples were conducted in the known range of Maui’s dolphins during two austral summers: 4 February – 2 March 2010 and 14 February – 10 March 2011. Seventy-three biopsy samples were collected during these surveys: 37 in 2010 and 36 in 2011. DNA profiles were completed for each sample, including genotyping of 20 variable microsatellite loci, genetic sex identification and mitochondrial (mt)DNA control region sequencing. These profiles were used to identify individual Maui’s dolphins and Hector’s dolphin migrants, to describe individual movements, and to estimate the abundance, population trend and effective population size of Maui’s dolphins for 2010–11, including comparison with data from a previous set of samples collected in 2001–07.

Based on the microsatellite genotyping, we identified 26 individuals from the 37 samples collected in 2010 (16 females, 10 males) and 27 individuals from the 36 samples collected in 2011 (16 females, 11 males). Twelve individuals were sampled in both 2010 and 2011, and with the addition of 1 unique beachcast male recovered in 2010, this provided a minimum census of 42 individuals (25 females, 17 males) alive at some point during the two years of the survey. Of this total, two females were identified as West Coast South Island Hector’s dolphin (*C. h. hectori*) migrants based on distinct mtDNA haplotypes and genotype-based population assignment procedures.

A minimum census of 89 individuals (49 females, 40 males) were sampled alive or dead along the west coast of the North Island at some point between January 2001 and March
2011. This total includes 35 Maui’s dolphins (18 females; 17 males) sampled alive in 2001–06; 32 Maui’s dolphins (18 females, 14 males) sampled alive in 2010–11; 7 Maui’s dolphins (5 females, 2 males) sampled alive in both 2001–06 and 2010–11; 13 Maui’s dolphins (6 females, 7 males) sampled dead between 2001 and 2011; and 2 female Hector’s dolphin migrants sampled alive in 2010–11.

Individual movements inferred from sampling locations in 2010 and 2011 were on a similar scale within and between years, spanning minimum straight-line distances up to 80.4 km, suggesting that at least some individuals move throughout a large portion of the current distribution of Maui’s dolphins. Mitochondrial (mt) DNA control region sequencing (360 bp) confirmed that 39 individuals represented the single unique haplotype (‘G’) diagnostic of Maui’s dolphin samples collected since 1988. The two Hector’s dolphin females sampled in 2010–11 represented haplotypes ‘I’ and ‘J’, which are common in populations along the west coast of the South Island.

The abundance and annual rate of change for Maui’s dolphins ≥ 1 year old was estimated using both closed- and open-population capture-recapture models based on DNA profiles. For 2010–11, abundance was estimated to be 55 individuals (95% CL = 48, 69), using a two-sample closed-population model. For the extended time period of 2001–11, an open-population Pradel Survival and Lambda model provided an estimate of annual survival of 84% (95% CL = 75%, 90%) and population decline of –3% per year (95% CL = –11%, +6%), although a downward or upward trend could not be confirmed with 95% confidence. The annual abundance estimates (N-hat) derived from a POPAN open-population model also suggest a small, but inconclusive, downward trend between 2001 and 2011. The effective population size (N_e), which estimates the effective number of breeding adults in the parental generation for the 2010–11 samples, was relatively large (N_e = 69, 95% CL = 31, 641) when compared with the capture-recapture estimate of abundance. This suggests that the population has likely experienced a recent decline, but has maintained a surprising, albeit low, level of genetic diversity given the small population size.
Our results highlight the importance of individual identification and genetic monitoring using biopsy samples and DNA profiling for better understanding dolphin population dynamics. The remarkable movement (≥ 400 km) of the two female Hector’s dolphins from the South Island’s west coast to the Maui’s dolphin population on the North Island’s west coast is the first documented contact between these two subspecies. While there is currently no evidence of mating between Hector’s dolphins and the Maui’s dolphins, this ‘natural translocation’ provides the potential for enhancing the low genetic diversity of the small Maui’s dolphin population.

Introduction

The critically endangered Maui’s dolphin (Cephalorhynchus hectori maui) is currently restricted to a relatively small stretch of coastline along the west coast of New Zealand’s North Island. This subspecies was classified as distinct from the Hector’s dolphin subspecies (C. h. hectori) on the basis of morphological differentiation and geographic and mitochondrial DNA isolation, having a single unique haplotype (‘G’) since at least 1988 (Baker et al. 2002, Pichler 2002, Hamner 2008). Using extrapolated rates of fisheries-related mortality and estimated life history parameters based on those of Hector’s dolphins, a population dynamic model suggested a substantial decline in the abundance of both Hector’s and Maui’s dolphins since the advent of nylon monofilament set nets in the late 1960s (Martien et al. 1999, Slooten et al. 2000). In 2001, the New Zealand Ministry of Fisheries began considering fishing restrictions to reduce the entanglement of these dolphins, and the most recent restrictions on set nets, drift nets and trawling in the core distribution of the Maui’s dolphin were enacted in 2008 (New Zealand Ministry of Fisheries 2008). Estimating and monitoring trends in abundance and effective population size are key factors for planning and evaluating continued actions to conserve the remnant population of Maui’s dolphins.
Capture-recapture analysis based on natural markings has proven to be a powerful method for the estimation of abundance in cetaceans. Unfortunately, Maui’s dolphins are difficult to individually identify based on natural markings, including scars or nicks, as less than 10% of individuals have distinctive markings (Gormley et al. 2005, Oremus et al. 2012). Even where individuals have distinctive markings, these can change over time and are often indistinguishable on beachcast animals, leading to ‘tag loss’. Individual identification by DNA profiling with microsatellite genotypes overcomes this problem, providing a permanent and heritable mark, suitable for a census or abundance estimate of populations, living or dead (Garrigue et al. 2004, Baker et al. 2007). The development of a lightweight biopsy dart, fired from a veterinary capture rifle, provides a low-impact method for collecting genetic samples from small cetaceans (Krützen et al. 2002). Together, biopsy sampling and genotyping provide a powerful approach to describing community structure and estimating abundance in small populations of dolphins (Oremus et al. 2007), as well as allowing larger-scale genetic monitoring (Schwartz et al. 2007), including estimates of the effective population size. Effective population size is an important parameter in conservation genetics that represents the number of effective breeding individuals in the parental generation, and determines the extent of loss in genetic diversity in the subsequent generation. Although not easy to estimate in species with overlapping generations, it is useful because it provides a better gauge for the loss of genetic diversity in a population and could be a better detector of population declines than monitoring abundance (Waples and Do 2008, Tallmon et al. 2010).

Our work continued the genetic monitoring of the Maui’s dolphin subspecies by using DNA profiles to estimate the current abundance and effective population size, as well as to document movements of individuals.
Objectives

- Archive Maui’s dolphin tissue samples collected in 2010 and 2011, in collaboration with Department of Conservation (DOC) personnel;
- Complete DNA profiles for all samples collected in 2010–11, including mtDNA control region sequence, genetic sex identification and microsatellite genotypes
- Identify additional variable microsatellite loci and genotype them for all samples collected in 2001–11 to increase confidence in individual identification
- Compile a census of individuals sampled in 2001–11
- Describe movements of individuals re-sampled in 2001–11
- Identify Hector’s dolphin migrants sampled among Maui’s dolphins in 2010–11
- Estimate Maui’s dolphin abundance for 2010–11
- Estimate Maui’s dolphin abundance and trends across 2001–11
- Estimate the effective population size ($N_e$) of Maui’s dolphins for 2010–11 and 2001–07 to provide a historical comparison

Methods

Sample collection

Skin biopsy samples were collected within the current known range of Maui’s dolphins during dedicated small boat surveys conducted by DOC during 4 February – 2 March 2010 and 14 February – 10 March 2011 (Oremus et al. 2012). Samples were collected using a small, lightweight biopsy dart (PaxArms NZ Ltd.) fired from a modified veterinary capture rifle, similar to that described by Krützen et al. (2002). Calves, approximately one-half or less the size of an adult and assumed to be less than 1 year old, were excluded from biopsy sampling.

Maui’s and Hector’s dolphin samples previously collected and archived at the University of Auckland Cetacean Tissue Archive were also utilised for individual identification, as a
reference dataset for population assignment, and a historical comparison for estimating Maui’s dolphin population trends. This included an additional 70 biopsy samples collected from Maui’s dolphins during small-boat surveys conducted from January 2001 to February 2006, 13 samples collected during the necropsy of Maui’s dolphins found beachcast or entangled in fishing gear between 2001 and 2010 (Baker et al. 2013), and 180 Hector’s dolphin samples collected around the South Island between 1988 and 2007 (Hamner 2008, Hamner et al. 2012).

**DNA extraction and genetic sex identification**

All samples were stored in 70% ethanol at −20°C prior to total cellular DNA extraction from a sub-sample using a standard Phenol/Chloroform/Isoamyl (PCI) protocol (Sambrook et al. 1989) as modified for small samples by Baker et al. (1994). The sex of each sample was identified using a multiplexed PCR protocol to amplify fragments of the sry and ZFX/ZFY genes (Gilson et al. 1998). The observed sex ratio of individuals was compared with an expected 1:1 sex ratio using a two-tailed exact binomial test with alpha set to 0.05. To assess the ability of the exact binomial test to reject the expected 1:1 ratio, a post hoc power analysis was conducted in G*Power 3.1.3 (Faul et al. 2007) using an effect size of 0.1. The minimum effect size that could be detected with 80% power using a sample size of 42 was also calculated.

**Mitochondrial DNA haplotypes**

Approximately 700 bp of the 5’ end of the mitochondrial (mt) DNA control region were amplified and prepared for sequencing according to (Hamner 2008). Sequencing was carried out using an ABI 3130 Genetic Analyzer (School of Biological Sciences, University of Auckland). Sequences were trimmed to align with 360 bp reference sequences of the single Maui’s dolphin haplotype (‘G’), as well as the 20 known Hector’s dolphin haplotypes (Pichler et al. 1998, Pichler and Baker 2000, Pichler 2002, Hamner 2008) using Geneious Pro 5.0.2 (Biomatters Ltd.).
**Individual identification**

Previous genotyping of Maui’s dolphins collected from 2001 to 2007 relied on 14 variable microsatellites (Baker et al. 2013). Given the low diversity for most of these loci and the increased sample size, an additional 11 loci were screened for variability in the Maui’s dolphin, and the 6 found to be variable were genotyped for all samples collected from 2001 to 2011 (Table IV.1). Each locus was amplified individually according to the conditions specified in Table IV.1, and co-loaded with up to five other loci amplified from the same individual for sizing by an ABI 3730 Genetic Analyzer (School of Biological Sciences, University of Auckland). GENEMAPPER v. 3.7 (Applied Biosystems) was used to bin and visually verify the resulting size peaks. Each amplification and sizing run included a negative control to detect contamination and ten internal control samples to standardise allele binning with previous genotyping runs and to estimate genotyping error, as recommended by Bonin et al. (Bonin et al.).

Microsatellite genotypes were compared for the purposes of individual identification, both within and across sampling years, using the program CERVUS 3.0.3 (Kalinowski et al. 2007). Initial comparisons allowed for mismatching of up to five loci (‘relaxed matching’) to prevent false exclusion due to genotyping error, particularly allelic dropout. Relaxed matches were visually examined for potential allelic dropout, as well as matching sex and mtDNA haplotype, and repeated up to three times to confirm or correct the genotype as necessary. After review and correction, samples with identical genotypes were accepted as resamples of the same individual (i.e. genotype captures and recaptures), based on a low probability of identity ($P_{ID}$) and probability of identity for siblings ($P_{ID\text{,sib}}$) as recommended by Waits et al. (Waits et al. 2001). For each locus, GenAlEx v6.4 (Peakall and Smouse 2006) was used to calculate $P_{ID}$, $P_{ID\text{,sib}}$, observed and expected heterozygosity, and to test for deviations from Hardy-Weinberg equilibrium.
**Movement of individuals**

Individual movements were documented by examining the sampling locations of replicate samples from the same individual. The straight-line distance between the coordinates of sampling locations was measured using a distance calculator available at http://jan.ucc.nau.edu/~cvm/latlongdist.html. None of the straight-line distances crossed land, so no modifications were required to follow the coastline. As the exact path taken by each dolphin is unknown, these measurements represent a minimum distance traveled over the time elapsed between sampling events.

**Subspecies identification and population assignment**

To confirm the unexpected discovery of mtDNA haplotypes ‘I’ and ‘J’ among the Maui’s dolphins (see section 4.5), the complete sample processing (DNA extraction through genotyping) was repeated independently twice by colleagues (A. Alexander and K. Thompson/E. Carroll). Identical results were produced by each of the three repetitions. The subspecies and population of origin for the two individuals having ‘I’ and ‘J’ mtDNA haplotypes were identified using a Bayesian assignment procedure implemented in *Structure* v2.3.2 (Pritchard et al. 2000, Pritchard et al. 2007) to compare 10-locus microsatellite genotypes for these samples to a reference dataset of 89 Maui’s and 180 Hector’s dolphins (East Coast South Island $n = 97$, West Coast South Island $n = 53$, South Coast South Island $n = 30$). The ‘Use PopInfo’ option (G = 0), with no population information included for the ‘I’ and ‘J’ haplotype individuals, was used to run $10^6$ Markov Chain Monte Carlo (MCMC) replicates following a burn-in of $10^5$ for $K = 4$ populations (Maui’s dolphin, East Coast South Island, West Coast South Island, South Coast South Island).
**Abundance, 2010–11**

Genotype recaptures were assembled into capture histories for individuals sampled in 2010–11. The Lincoln-Petersen estimator with Chapman correction (Chapman 1951) is the only model available for estimating abundance in this two-sample design. This model assumes that:

- the population is geographically and demographically closed
- all animals are equally likely to be sampled in each occasion
- tags are permanent and read correctly

Previous studies showed that the Maui’s dolphin population is geographically isolated and has no gene flow with Hector’s dolphin populations (Pichler et al. 1998, Pichler 2002, Hamner et al. 2012). Although the strict assumption of a demographically closed population is violated for most studies of wild populations, the short two-year time span of our study minimises the potential for births or deaths in the population. Only biopsy-sampled individuals were included in the abundance analyses, as beachcast individuals were unavailable for recapture after recovery. Along with the exclusion of calves from biopsy sampling, this means that our abundance estimate applies to the living population of individuals approximately ≥ 1 year old (see Webster et al. 2010 for a collation of available age-length relationships in Hector’s and Maui’s dolphins). Individual identification by DNA profiling provides a permanent tag, and the use of controls and rigorous genotype error checking procedures minimise the potential for incorrectly reading the genotype tag (see section 4.2). Consequently, we consider that our dataset is robust with respect to the assumptions of the Chapman corrected Lincoln-Petersen estimator, and it was applied according to the following formula:

\[ N = \frac{(n_1+1)(n_2+1)(m_2+1)}{m_2+1} - 1 \]

where  
\( N \) = abundance  
\( n_1 \) = number of individuals sampled in occasion 1  
\( n_2 \) = number of individuals sampled in occasion 2  
\( m_2 \) = number of individuals sampled in both occasions 1 and 2
A 95% confidence interval (CI) was calculated according to Chao’s (Chao 1989) method for sparse data:

\[
\text{Lower 95\% CL} = M_{k+1} + f_0^*/C \\
\text{Upper 95\% CL} = M_{k+1} + f_0^*\cdot C
\]

where \( M_{k+1} \) = the total number of distinct animals ‘captured’ during the study

\[
f_0^* = N - M_{k+1}
\]

\[
C = \exp\{1.96[\log(1+(\text{var}^*(N)/f_0^2))]^{1/2}\}
\]

\[
\text{var}^*(N) = [(n_1+1)(n_2+1)(n_1-m_2)(n_2-m_2)]/[m_2+1]^2(m_2+2)
\]

**Population trend, 2001–11**

Genotype recaptures were assembled into capture histories for individuals sampled across the entire period from 2001 to 2011. Only biopsy-sampled individuals were included in these analyses, as beachcast animals are unavailable for recapture after recovery, and would therefore confound the estimated probability of capture. A goodness of fit test was carried out in U-CARE v2.02 (Choquet et al. 2009) to assess the fit of the data to a general Cormack-Jolly-Seber framework and assess whether issues of transients (animals passing through the study area, but not likely to remain in the area to be available for subsequent sampling) or ‘trap-dependence’ (an increase or decrease in the likelihood of an individual to be re-sampled after the first sampling) were likely to confound our analyses.

**Pradel Survival and Lambda**

To estimate the annual rate of change in the Maui’s dolphin population, eight candidate models were run using the Pradel Survival and Lambda framework in MARK v5.1 (White and Burnham 1999). These models included all combinations of constant (.) and time variable (t) conditions for the three parameters: survival (\( \phi_i \)), recapture probability (\( p \)), and annual rate of change (\( \lambda \)). Candidate models were evaluated using Akaike’s
Information Criterion corrected for small sample sizes (AICc) and delta AICc, which represents the difference between the AICc for a given model and the lowest AICc (e.g. the model with the lowest AICc has a delta AICc of 0). The best model was selected based on having the lowest AICc and a delta AICc > 2 when compared with the model having the next lowest AICc, according to the rule of thumb given by (Burnham and Anderson 2002).

**POPAN**

Estimates of abundance (\(N\)-hat) for each of the seven sampling years between 2001 and 2011 were derived from the best model using the open-population POPAN framework in MARK v5.1 (White and Burnham 1999). Eight candidate models were run, which included all combinations of constant (.) and time variable (t) for the three parameters: survival (\(\phi\)), recapture probability (\(p\)) and probability of entry (\(p_{ent}\)). As for the Pradel analysis described above, the best model was selected based on having the lowest AICc score and a delta AICc > 2 when compared with the model having the next lowest AICc.

**Effective population size**

Effective population size (\(N_e\)) was estimated using the linkage disequilibrium method implemented in LDNe (Waples and Do 2008). With this model, the estimate of \(N_e\) represents the number of breeding individuals in the parental generation of the sample. This method was applied to the samples collected in 2010–11, as well as those from 2001–07 to act as a historical comparison, acknowledging that there is generational overlap within and between the two time periods. The locus EV37 was excluded from the genotypes for this analysis as it showed evidence of null alleles and a highly significant deviation from Hardy-Weinberg equilibrium across all time periods. Although the presence of null alleles will not affect the individual identification, it could bias the estimate of \(N_e\). The two Hector’s dolphin migrants were also excluded from this analysis, as this method assumes no migration and there is currently no evidence that these two females are part of the current breeding population or were part of the breeding
population that produced the sampled generation. Therefore, a set of 19-locus genotypes was used to calculate $N_e$ for 2010–11 ($n = 40$) and 2001–07 ($n = 54$), excluding alleles with frequencies less than 0.02, as recommended by Waples and Do (2010).

**Results**

**Sample collection**
A total of 73 skin biopsy samples were collected during dedicated small-boat surveys conducted during 4 February – 2 March 2010 ($n = 37$) and 14 February – 10 March 2011 ($n = 36$) between Kaipara Harbour to New Plymouth (Figure IV.1; Table IV.2; Appendices 1 and 2). One sample was also collected during the necropsy of a Maui’s dolphin found beachcast at Raglan on 20 November 2010.

**Individual identification**
Each sample was genotyped for up to 20 variable microsatellite loci, with an average of 19 loci per sample (Table IV.3). The number of alleles for each variable locus was low, ranging from 2 to 7 alleles (2 to 9 alleles when including Hector’s migrants). Based on the repeated genotyping of the 10 control samples (252 alleles), the initial genotyping error rate was 0.004; however, the final error rate will be less than this, as additional replicates were completed to confirm or correct genotypes of ‘relaxed matches’. The overall probability of identity ($P_{(ID)}$) was $1.7 \times 10^{-7}$ and probability of identity for siblings ($P_{(ID)_{sib}}$) was $5.6 \times 10^{-4}$ (Table IV.3). Given this low probability of a match by chance and the small size of the population, unique genotypes were considered to be unique dolphins, and samples with matching genotypes were considered replicate samples (i.e. genotype recaptures) of the same individual. Sex and mtDNA haplotype were subsequently compared and agreed for all of the genotype matches.
**Minimum census and sex of individuals**

*2010–11.* From the 37 biopsy samples collected in 2010, 26 individuals were identified (16 females, 10 males), of which 17 were sampled once, 7 were sampled twice, and 2 were sampled three times. From the 36 biopsy samples collected in 2011, 27 individuals were identified (16 females, 11 males), of which 18 were sampled once and 9 were sampled twice. Twelve individuals were biopsy sampled in both 2010 and 2011, providing a total of 41 individuals sampled during the 2010 and 2011 surveys. The one male beachcast sample collected in 2010 did not match any of the biopsy-sampled individuals, increasing the total to a minimum census of 42 individuals (25 females, 17 males) sampled alive or dead during 2010–11.

*2001–11.* The comparison of genotypes from the 42 individuals sampled during 2010–11 with 43 individuals biopsy sampled during the 2001–06 surveys and 12 individuals sampled after death between 2001 and 2007 revealed seven individuals that were first sampled during the 2001–06 surveys and sampled again in the 2010–11 surveys. Therefore, a minimum census of 89 individuals (49 females, 40 males) were sampled alive or dead along the west coast of the North Island at some point from January 2001 to March 2011. This total includes 35 Maui’s dolphins (18 females; 17 males) sampled alive in 2001–06; 32 Maui’s dolphins (18 females, 14 males) sampled alive in 2010–11; 7 Maui’s dolphins (5 females, 2 males) sampled alive in both 2001–06 and 2010–11; 13 Maui’s dolphins (6 females, 7 males) sampled after death between 2001 and 2011; and 2 female Hector’s dolphin migrants sampled alive in 2010–11 (see section 4.5).

**Sex ratio.** No statistically significant difference from a 1:1 sex ratio was found for the total individuals or for any of the sampling periods or types (Table IV.4). However, the power of this test to detect an effect size of 0.1 was low (Table IV.4), and only a skewed sex ratio with an effect size larger than 0.22 would be detectable with 80% power using a sample size of 42.
Movement of individuals

The locations of biopsy samples collected in 2001–06 are known only to the level of their primary survey strata (i.e., north of Manukau, south of Manukau, north of Port Waikato, south of Port Waikato; Baker et al. 2010). However, even these limited data can provide information on the movements of individual dolphins over the entire study period. Of the individuals sampled more than once between 2001 and 2011, but having at least one sample without a precise location, 11 were re-sampled 2–5 times in the same strata, and 8 were resampled 2–5 times in two to three different strata. These re-samples indicate some local site fidelity, as well as movements by some individuals across the Manukau Harbour entrance and the mouth of the Waikato River. This pattern is similar to that obtained from the more detailed analysis of dolphin movements carried out in 2010–11.

Movements by individuals within and between the 2010 and 2011 survey periods were documented by examining the precise sampling locations of replicate samples from the same individuals (Table IV.5; Figure IV.2; Oremus et al. 2012). Distances between re-samples within 2010 ranged from 0.65 km for an individual re-sampled within an hour to 26.44 km for an individual sampled south of Manukau and then north of Raglan five days later. Distances between re-samples within 2011 ranged from 0.32 km within 13 minutes to 78.62 km for an individual sampled in South Manukau and then in North Manukau 19 days later.

Movements of individuals between the 2010 and 2011 sampling periods were of a similar scale to within-year movements, ranging from 0.88 km over 372 days to 80.43 km over 375 days (Table IV.3). The individual (NI10-21) sampled across the largest distance showed interesting movements both within and between years. In 2010, she was sampled twice across 11.33 km over 2.5 hours to the south of the Kaipara Harbour. A little over one year later, she was sampled about half way between Manukau Harbour and the mouth of the Waikato River, 80.43 km south of her previous sampling location, before she returned 78.62 km within 19 days to be sampled again in nearly the same location as she was sampled in 2010.
Mitochondrial DNA haplotypes and identification of migrants

Sequencing of an mtDNA control region fragment confirmed that 39 of the 41 individuals sampled in 2010 and 2011 were haplotype ‘G’, the only haplotype detected in samples of Maui’s dolphins between 1988 and 2007. The other two individuals represented haplotypes ‘I’—individual NI10-03 sampled in 2010, and ‘J’—individual NI10-24 sampled in both 2010 and 2011 (Table IV.2). NI10-03 and NI10-24 were clearly assigned as Hector’s dolphins from the West Coast South Island population based on population assignment using a reference dataset of 10 microsatellite loci for both subspecies (Figure IV.3).

Abundance, 2010–11

Recapture histories for the individuals biopsy sampled in 2010–11 (including the two Hector’s dolphin migrants) were used to calculate an abundance of $N = 57$ (95% CL = 49, 71) for the individuals approximately ≥ 1 year old. This estimate is consistent with the 2011 abundance estimate produced by the POPAN model described in the following section. When the two Hector’s dolphin migrants were removed from the calculation, the abundance estimate decreased slightly to $N = 55$ (95% CL = 48, 69).

Population trend, 2001–11

The goodness of fit test found no significant deviation from the general model ($p = 0.860$). There was also no evidence for transients ($p = 0.529$), confirming that individuals are not likely to be just passing through the study area, or for ‘trap-dependence’ ($p = 0.138$), indicating that the act of sampling an individual does not make it more or less likely to be re-sampled in the future.

Pradel survival and lambda.

Of the eight candidate models run, $\phi(t)p(t)\lambda(t)$ was selected as the best model based on the lowest AICc score and a delta AICc of 4.52 when compared with the next best model (Table IV.6a). This model produced estimates for all
three parameters, with the annual rate of change ($\lambda$) estimated to be 0.97 (95% CL = 0.89, 1.06; Table IV.6b). While this suggests that the population declined by 3% per year during 2001–11, a decline cannot be confirmed with 95% confidence. This model also estimated annual survival ($\phi$) to be 0.83 with reasonable precision (95% CL = 0.75, 0.90), suggesting an annual mortality rate of 17% per year for age $1^+$ dolphins. This survival estimate is in the middle of the range of values previously reported for ≥ 1 year old Hector’s dolphins: 0.77–0.89 (Slooten and Lad 1991, Slooten et al. 1992, Slooten and Dawson 1994, Cameron et al. 1999). The probability of genotype capture for an individual ($p$) varied from year to year, between 0.04 and 0.53, and was consistent with annual sampling effort and sample sizes (Table IV.6b).

**POPAN.** Of the eight candidate models run using POPAN, $\phi(t)p(t)pent(.)$ was selected as the best fit based on having the lowest AICc score and a delta AICc of 8.74 when compared with the next best model (Table IV.7a). The POPAN model produced estimates of survival ($\phi= 0.84$, 95% CL = 0.75, 0.90) and annual probability of capture ($p$ ranging from 0.05 to 0.57; Table IV.7b) similar to the Pradel analysis above. However, as these two analyses have the same underlying framework, this agreement should not be interpreted as independent verification of the estimates. The abundance estimates derived for each year ($N$-$\text{hat}$) range from 45 to 71 and exhibit an overall downward trend, with an $N$-$\text{hat}$ for 2011 of 52 (95% CL = 30, 73) (Table IV.7c).

**Effective population size**

The effective population size ($N_e$) calculated for the 2001–07 sample was $N_e = 75$ (95% CL = 36, 368) and for 2010–11 was 69 (95% CL = 31, 641). Although there is a slight decline in the point estimates between these two periods, they have wide and overlapping confidence intervals.
Discussion

Our work demonstrated the utility of genetic monitoring for estimating both demographic and genetic population parameters for the Maui’s dolphin. The vessel surveys were highly successful in collecting biopsy samples from 41 individuals: 39 Maui’s dolphins and 2 Hector’s dolphin migrants.

Excluding the Hector’s dolphin migrants, the 2010–11 Maui’s dolphin abundance was estimated to be approximately 55 individuals. The exclusion of calves from biopsy sampling is not likely to bias our results given the small number of calves that were sighted, however, the estimates reported here should be interpreted as applying to the portion of the population $\geq 1$ year old. Although not directly comparable given the different methods used, our Maui’s dolphin abundance estimate is considerably lower than estimates made in the period from 1985 to 2004, which were calculated from vessel and aerial line-transect surveys and ranged from 75 to 140 individuals (Dawson and Slooten 1988, Martien et al. 1999, Russell 1999, Ferreira and Roberts 2003, Slooten et al. 2006). Our current estimate was also lower than the estimate of 80 (95% CL = 42, 152) produced by a Pradel-like genotype recapture analysis of samples collected in 2001–07 (Baker et al. 2013), although the confidence intervals are largely overlapping. The biopsy samples from the 2001–07 data were included in our direct assessment of the population trend, and although we did not find conclusive evidence for a decline in the Maui’s dolphin population, our analysis does suggest that a small decline is likely. It is important to note that the power to detect a decline decreases as population size decreases (Taylor and Gerrodette 1993), and that our results do not offer conclusive evidence that the population is not declining. Despite its small size, the Maui’s dolphin population appears to be maintaining an equal sex ratio, or potentially a slight female bias, which would presumably be favorable for reproduction.

The low estimates for both abundance and effective population size are consistent with a demographic bottleneck within the past few generations. The similar size of the two
estimates, however, is puzzling as effective population size is generally lower than abundance (Frankham 1995). Although the affect of overlapping generations on the LDNe estimator lacks a rigorous evaluation (Waples 2006, Waples and Do 2008), the potential bias is likely to underestimate rather than overestimate the true effective population size (Luikart et al. 2010). The larger effective population size relative to abundance is consistent with a recent decline, but suggests that the Maui’s dolphin is maintaining a surprising level of genetic diversity given its small population size (Crandall et al. 1999). However, the genetic diversity of Maui’s dolphins is low compared with Hector’s dolphins (Hamner 2008) and their long generation time—estimated to be 12.5 years (Taylor et al. 2007)—is likely to be buffering the population from a more severe loss of genetic diversity. Similar patterns have been observed in a variety of endangered species reduced to small numbers, including the greater one-horned rhinoceros (Dinerstein and McCracken 1990), white-tailed eagle (Hailer et al. 2006) and copper redhorse (a fish; Lippe et al. 2006). The estimated 12.5 year generation time for Maui’s dolphins means that a subtle change in effective population size is unlikely to be detected across the short time period between our two sample sets.

The surprising movement (\(\geq 400\) km) of the two female Hector’s dolphins from the West Coast South Island population to the Maui’s population is the first documented contact between these two subspecies. As they are both female, there is the potential for the ‘I’ and ‘J’ haplotypes to persist in the Maui’s dolphin population via maternal inheritance. While there is currently no evidence of mating between these Hector’s dolphin migrants and the Maui’s dolphins, this ‘natural translocation’ provides the potential for enhancing the low genetic diversity of the Maui’s dolphin population. Although we prefer to be optimistic about the potential for spiking the shallow gene pool of the Maui’s dolphin, there is also the potential for outbreeding depression, where local adaptations are lost in ‘hybrid’ offspring, causing them to be less fit than individuals of either ‘pure’ subspecies (Marr et al. 2002). The expansion of genetic monitoring efforts to genomic level analyses and functional loci (e.g., MHC) could shed light on any local adaptations these subspecies might have developed.
Genotype recaptures allowed the observation of record individual movements by Maui’s dolphins—up to 80 km within their known range. As one dolphin travelled 78 km over a period of just 19 days, individual home ranges of Maui’s dolphins may be larger than is currently inferred from the estimated home range of Hector’s dolphins around Banks Peninsula (Rayment et al. 2009). This means that at least some Maui’s dolphins are utilising a large portion of the current distribution of the subspecies, rather than a restricted localised home range. These large movements within the Maui’s distribution, along with the discovery of the Hector’s dolphin migrants, suggest the need for protecting corridors within and between core distributions of Maui’s and Hector’s dolphins.

After the conclusion of our surveys and primary genetic analyses, the carcass of an adult female dolphin was recovered on Clark’s Beach inside the Manukau Harbour on 26 October 2011. At the time of this report, genetic analysis of this sample to confirm its subspecies identity has not been completed, but it was identified as a reproductively mature female (New Zealand Department of Conservation 2011). Another dolphin was incidentally caught in a set net off Taranaki on 2 January 2012 (Ministry of Agriculture and Forestry 2012). Unfortunately, no genetic sample was collected from the carcass and its subspecies identity is unknown.

Our results highlight the importance of individual identification and genetic monitoring using biopsy samples and DNA profiling, particularly for morphologically indistinguishable subspecies or populations. Continued genetic monitoring over informative time scales is recommended as part of the Maui’s dolphin recovery programme. Only time and genetic monitoring will reveal if the Hector’s dolphin migrants remain and breed successfully with the Maui’s dolphins. Our census of known individuals and their 2001–11 capture histories will provide an excellent resource for documenting the deaths of any known individuals from recovered carcasses, monitoring the minimum longevity of known individuals, and as a foundation for future genotype recapture analysis and genetic monitoring.
Acknowledgments

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References


Pritchard, J. K., X. Wen and D. Falush. 2007. Documentation for *structure* software:


Table IV.1. Twenty variable microsatellite loci genotyped in samples of Maui's dolphins ($n = 151$) and Hector's dolphin migrants ($n = 5$) collected 2001–11, and five loci that were found to be monomorphic (shaded gray). ‘S Gui’ loci were amplified according the protocol of Cunha and Watts (2007) with the annealing temperatures ($T_A$) listed*, and all other loci were amplified in 10µL reactions containing 1x PCR II buffer, 1.5 mM MgCl2, 0.4 µM each primer, 0.2 mM dNTP, 0.125 units Platinum Taq (Invitrogen) and 10–20 ng/L DNA template, and run with locus-specific annealing temperatures ($T_A$) in the following thermocycling profile: 93ºC for 2 min; (92ºC for 30 s, $T_A$ for 45 s, 72ºC for 50 s) x 15; (89ºC for 30 s, $T_A$ for 45 s, 72ºC for 50 s) x 20; 72ºC for 3 min.

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<th>Primer Source</th>
<th>Label</th>
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<th>$n$</th>
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<th># alleles in Maui's</th>
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Table IV.2. Biopsy samples collected during Maui’s dolphin surveys conducted (a) 4 February – 2 March 2010 and (b) 14 February-10 March 2011 (Oremus et al. 2012). The sample code prefix ‘Chem’ refers to Maui’s dolphins (*Cephalorhynchos hectori maui*) and ‘Che’ refers to those subsequently identified as Hector’s dolphins (*C. h. hectori*).

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<th>Sample Code</th>
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<th>Latitude (°S)</th>
<th>Longitude (°E)</th>
<th>Location</th>
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Table IV.3. Twenty variable microsatellite loci genotyped for Maui’s dolphins and Hector’s dolphin migrants sampled in 2001-11. Observed (Ho) and expected (He) heterozygosity are shown along with a test of deviation from Hardy-Weinberg equilibrium (HWE \( p \); \( p < 0.05 \) are bold). \( n \) = number of samples; \# indiv = number of individuals after removal of replicates, \( k \) = number of alleles.

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Overall 74 42 3.3 69 40 2.8 82 54 2.6 156 1.7x10^{-7} 5.6x10^{-4}
Table IV.4. Sex of Maui’s dolphin and migrant Hector’s dolphin individuals sampled from January 2001 to March 2011. †Includes an individual biopsied alive, and found beachcast two years later. ‡Includes two Hector’s dolphin migrants. A two-tailed binomial distribution test was used to assess significant deviation from a 1:1 sex ratio ($p < 0.05$), and the associated power ($1-\beta$) to detect an effect size of 0.1 is reported.

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Table IV.5. Individual movements of Maui’s dolphins and a Hector’s dolphin migrant (\(^\star\)) that were sampled more than once during 2010–11, as identified by genotype recapture. Samples from the same individual are grouped in alternating shaded and non-shaded blocks with the ID code in bold (an individual’s first sample code is used as its ID code). Distances observed between recapture locations (‘Distance (km)’) within and across years were measured as straight-line distances using the distance calculator (http://jan.ucc.nau.edu/~cvm/latlongdist.html). *Sample pair used for calculating the maximum straight-line distance between recaptures.

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<th>Date</th>
<th>Location</th>
<th>Latitude (°S)</th>
<th>Longitude (°E)</th>
<th>Sex</th>
<th>Within 2010 Distance (km)</th>
<th>Time Span</th>
<th>Within 2011 Distance (km)</th>
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Table IV.6. (a) Eight candidate models run using Pradel Survival and Lambda framework in MARK v5.1 for Maui’s dolphins and Hector’s dolphin migrants biopsy sampled in 2001-11, where (t) means the parameter was allowed to vary between occasions and (.) means it was held constant. (b) Survival ($phi$), capture probability ($p$) and annual rate of change ($lambda$) estimates from the best (bold) of the eight candidate models.

(a)

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<th>AICc Weights</th>
<th>Model Likelihood</th>
<th>Num. Par</th>
<th>Deviance</th>
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(b) $phi(.)p(t)lambda(.)$

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<th>95% CL</th>
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Table IV.7. (a) Eight candidate models run using the POPAN framework in MARK v5.1 for Maui’s dolphins and Hector’s dolphin migrants biopsy sampled in 2001-11, where (t) means the parameter was allowed to vary between occasions and (.) means it was held constant. (b) Survival (phi), capture probability (p) and probability of entry (pent) estimates from the best (bold) of the eight candidate models. (c) Annual abundance estimates (N-hat) derived from the best model.

(a) | Model | AICc | Delta AICc | AICc Weights | Model Likelihood | Num. Par |
--- | --- | --- | --- | --- | --- |
phi(.)p(t)pent(.) | 206.6434 | 0 | 0.97758 | 1 | 10 |
phi(.)p(t)pent(t) | 215.3847 | 8.74130 | 0.01236 | 0.0126 | 15 |
phi(t)p(t)pent(.) | 215.8477 | 9.20430 | 0.00981 | 0.0100 | 15 |
phi(t)p(t)pent(t) | 223.2470 | 16.6036 | 0.00024 | 0.0002 | 19 |
phi(t)p(.)pent(t) | 230.4329 | 23.7895 | 0 | 0 | 9 |
phi(.)p(t)pent(.). | 232.2461 | 25.6027 | 0 | 0 | 9 |
phi(t)p(.)pent(.). | 243.1019 | 36.4585 | 0 | 0 | 9 |
phi(.)p(.)pent(.). | 254.3225 | 47.6791 | 0 | 0 | 4 |

(b) phi(.)p(t)pent(.)

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<th>95% CL</th>
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(c) phi(.)p(t)pent(.)

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Figure IV.1. Location of biopsy samples collected during Maui’s dolphin surveys conducted (a) 4 February to 2 March 2010 \((n = 37)\) and (b) 14 February to 10 March 2011 \((n = 36)\). Maps taken directly from Oremus et al. 2010 and Oremus et al. 2011.
Figure IV.2. Movements of individuals identified by genotype recaptures (linked by black lines, or circles enclosing nearby resamples) during Maui’s dolphin surveys conducted (a) 4 February to 2 March 2010 ($n = 37$) and (b) 14 February to 10 March 2011 ($n = 36$). Maps from Oremus et al. 2010 and Oremus et al. 2011, modified to illustrate recapture locations.
Figure IV.3. Assignment of individuals to the Maui’s dolphin or East, West or South Coast Hector’s dolphin populations based on the *Structure* v.2.3.2 analysis of 11-locus microsatellite genotypes. Each vertical bar represents an individual and is shaded according to its coefficient of membership to the Maui’s (orange), East Coast (red), West Coast (blue) and South Coast (green) Hector’s dolphin populations. NI10-03 (haplotype ‘I’) and NI10-24 (haplotype ‘J’) were sampled in the Maui’s dolphin distribution, but are assigned with the highest probability to the West Coast, South Island population of Hector’s dolphins.
Appendix V

Tissue and Data Archiving

Tissue, DNA profiles and genotype recapture records used for the research presented in this dissertation have been archived to facilitate long-term genetic monitoring.

Tissue and Extracted DNA

Skin samples from Maui’s and Hector’s dolphins that were collected as part of my PhD project were sub-sampled for DNA extraction. The remaining tissue and extracted DNA were archived alongside samples previously collected from Maui’s and Hector’s dolphins in the New Zealand Cetacean Tissue Archive, curated by Rochelle Constantine at the University of Auckland.

DNA Profiles and Recapture Datasets

The files listed below are archived with the author, as well as the Cetacean Conservation and Genetics Laboratory at Oregon State University’s Hatfield Marine Science Center. Requests for data access can be submitted to Rebecca M. Hamner (rmhamner@gmail.com) and C. Scott Baker (scott.baker@oregonstate.edu), and should be accompanied by a proposal describing how the data would be used. A two-year embargo applies to the data, however, requests made before two years will be considered on a case-by-case basis, depending upon the proposed use and potential for collaboration.

DNA profiles for Maui’s dolphins 2001-2007

File name: DNA Profiles_Maui’s 01-07_RMH_18Apr14.txt

DNA profiles (sex, 576 bp mtDNA control region haplotype, ≤26 microsatellites) for Maui’s dolphins (Cephalorhynchus hectori maui; n = 82, representing 54 individuals) sampled along the west coast of New Zealand’s North Island between 2001 and 2007. The microsatellite genotypes include 14 loci from Baker et al. (2013) and were extended up to 26 loci here.
DNA profiles for Maui’s dolphins 2010-2011

File name: DNA Profiles_Maui’s 10-11_RMH_18Apr14.txt

DNA profiles (sex, 576 bp mtDNA control region haplotype, \( \leq 26 \) microsatellites) for Maui’s dolphins (\textit{Cephalorhynchus hectori maui}; \( n = 69 \), representing 40 individuals) sampled along the west coast of New Zealand’s North Island between 2010 and 2011. Twenty microsatellite loci were included in Oremus \textit{et al.} (2012; Appendix III), 21 loci were included by Hamner \textit{et al.} (in press; Chapter 3), and genotypes were extended up to 26 loci here.

DNA profiles for Hector’s dolphins in Cloudy Bay 2011-2012

File name: DNA Profiles_Hector’s CB11-12_RMH_18Apr14.txt

DNA profiles (sex, 576 bp mtDNA control region haplotype, \( \leq 26 \) microsatellites) for Hector’s dolphins (\textit{Cephalorhynchus hectori hectori}; \( n = 263 \), representing 148 individuals) sampled in Cloudy Bay, on the northeast corner of New Zealand’s South Island in February of 2011 and 2012.

DNA profiles for Hector’s dolphin subspecies sampled on the North Island 2005-2012

File name: DNA Profiles_Hector’s Subspecies NI_RMH_18Apr14.txt

DNA profiles (sex, 576 bp mtDNA control region haplotype, \( \leq 26 \) microsatellites) for the six Hector’s dolphins (\textit{Cephalorhynchus hectori hectori}) that were sampled on the North Island between 2005 and 2012, as described in Hamner \textit{et al.} (2014; Chapter 2).

Genotype recapture records for Maui’s dolphins 2001-2011

File name: Recapture Records_Maui’s 01-11_RMH_18Apr14.txt
    Recapture Records_Maui’s 01-11_RMH_18Apr14.pdf (formatted, color)

Genotype recapture records for Maui’s dolphin individuals (\( n = 89 \)) sampled along the west coast of New Zealand’s North Island between 2001 and 2011.

Genotype recapture records for Hector’s dolphins in Cloudy Bay 2011-2012

File name: Recapture Records_Hector’s CB 11-12_RMH_18Apr14.txt
    Recapture Records_Hector’s CB 11-12_RMH_18Apr14.pdf (formatted, color)

Genotype recapture records for Hector’s dolphin individuals (\( n = 148 \)) sampled in Cloudy Bay, New Zealand, in February of 2011 and 2012.
Reference genotypes for Hector’s and Maui’s dolphins 1988-2007

Previously published Hector’s and Maui’s dolphin genotypes (n = 229 individuals; Hamner et al. 2012) used as the reference database for individual assignment and identification of migrants, with one additional locus genotyped for the Maui’s dolphins as part of the current work.

References


