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**Population Structure and Genetic Variation  
in Hector's dolphin  
(*Cephalorhynchus hectori*)**

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**A thesis presented in partial fulfillment of the degree of  
Doctorate of Philosophy in Biology  
at the University of Auckland  
Auckland, New Zealand**

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**Preface:**

**STEWED FILLETS OF PORPOISE**

*Filets de Marsouin en daube*

When mounted on the bowsprit of a cutter you have harpooned a porpoise in the English Channel, open it lengthwise and take from it some nice fillets of fish.

Scald them, stick them with *lardons*, and let them brown in a pot with oil, garlic, onion, shallot, and flour; moisten with half a litre of water and half a litre of red wine; add salt, pepper, nutmeg, pimento, clove, and a *bouquet garni*; let it simmer on a small fire; add carrots and potatoes.

Skim before serving.

Henri de Toulouse-Lautrec and Maurice Joyant

*L'Art de la Cuisine*

Translated to English in 1966: The Art of Cuisine

P56

**Dedication:**

I dedicate this thesis to my wife, Victoria, without whose support, this thesis would never have been.

I further dedicate this thesis to my parents, Franz Xaver Pichler and Jennifer Susan Cranwell Pichler.

## Abstract:

This thesis uses molecular genetics as a tool to uncover information about the population structure and genetic variation in Hector's dolphin (*Cephalorhynchus hectori*), to track population declines and to assess the evolutionary origins and taxonomic status of this species. A high-resolution genetic analysis of population structure was considered important for the determination of population boundaries and delimitation of conservation management units due to potentially unsustainable fisheries-related mortality.

Population structure and dispersal rates were assessed using 281 samples collected from individual Hector's dolphins of ten population groups representing the known geographic range of this species. Variation among mitochondrial DNA sequences ( $\Phi_{ST} = 0.545$ ) and microsatellite allele frequencies at six loci ( $R_{ST} = 0.252$ ) indicated the presence of four genetically isolated regional populations, North Island ( $n = 29$ ), East Coast South Island ( $n = 110$ ), West Coast South Island ( $n = 122$ ) and South Coast South Island ( $n = 19$ ). Significant levels of genetic differentiation were not detected within local sub-populations of the East Coast and West Coast regional populations. However, the estimated geneflow between these sub-populations fitted a one-dimensional stepping-stone model ( $r^2 = 0.6225$ ) suggesting a vulnerability of local populations to fragmentation. A measure of expected mtDNA diversity (Tajima's  $D$  statistic) suggested decline in eight of the ten populations. Microsatellite heterozygosity was also lower than expected in the East Coast and North Island regions, suggesting either further regional sub-structuring (Wahlund effect), loss of diversity due to population decline or the presence of null alleles.

Examination of all Hector's dolphin museum specimens of known origin ( $n = 55$ ) enabled comparison of historic (1870 - 1987) genetic diversity to contemporary (1988 - 1999) diversity in two regional populations to assess the possibility that these populations have undergone recent declines. Over the last 20 years the North Island population has been reduced from at least three lineages ( $h = 0.41$ ) to a single lineage ( $h = 0$ ,  $p < 0.05$ ). The diversity of the East Coast, South Island population has declined significantly from  $h = 0.65$  to  $h = 0.35$  ( $p < 0.05$ ). These results suggest that the low abundance currently observed is due to recent population declines and that the North Island population is threatened with extinction in the near future. Based on a trend analysis of the mtDNA, it can be predicted that the East Coast South Island population may lose all mtDNA diversity within the next 20 years. Alternatively, detection of a one dimensional dispersal pattern may indicate that some populations are at risk of extirpation while others may not be in decline. If this is the case then the East Coast regional population is at risk of fragmentation.

On a wider evolutionary scale, Hector's dolphin is one of four species of the genus *Cephalorhynchus*, all of which suffer fisheries-related mortality. To describe the origin and radiation of these species, 485 bp of the mitochondrial DNA control region was sequenced from 320 individuals (including previously sequenced 200 Hector's dolphins) representing nine of the ten species in the sub-family Lissodelphininae. The hypotheses that either *Cephalorhynchus* is a monophyletic genus or that the four species have arisen separately from pelagic Lissodelphine species and have converged morphologically were tested. The mtDNA phylogeny supported the monophyly of the genus and suggested that the genus *Cephalorhynchus* originated in the waters of

South Africa and, following the West Wind Drift, colonised New Zealand and then South America. Secondary radiations resulting in two genetically isolated populations were found for the Kerguelen Island Commerson's dolphin and the North Island Hector's dolphin.

A comparison of the genetic differentiation between the Commerson's dolphins of the Kerguelen Islands ( $n = 11$ ) and the coast of South America ( $n = 35$ ), and between the North Island ( $n = 14$ ) and South Island ( $n = 185$ ) Hector's dolphins, was conducted in order to assess the conservation and taxonomic status of these populations. A single fixed substitution in the mtDNA control region was diagnostic for the Kerguelen Island compared to South America ( $F_{ST} = 0.306$ ,  $\Phi_{ST} = 0.602$ ) and the North Island compared to the South Island ( $F_{ST} = 0.442$ ,  $\Phi_{ST} = 0.495$ ). Population differentiation of four microsatellite alleles (including unique alleles in each of the four populations) between the Kerguelen Island and South American Commerson's dolphin ( $F_{ST} = 0.036$ ,  $R_{ST} = 0.0493$ ) and between the North and South Island Hector's dolphins ( $F_{ST} = 0.391$ ,  $R_{ST} = 0.3197$ ) indicated restricted nuclear as well as maternal gene flow. These data, combined with additional evidence of morphological and geographic isolation, indicated that the Kerguelen Island Commerson's dolphin and the North Island Hector's dolphin are likely to be reproductively isolated from their alternate con-specific populations. Examination of various species concepts and definitions of conservation units leads to the conclusion that these four populations should each be considered unique at the subspecies level for the purposes of management, protection and evolutionary potential.

These results lead to the conclusion that the Hector's dolphin consists of highly subdivided populations. As a result of this and a low reproductive potential, Hector's dolphin populations are vulnerable to extirpation through even low levels of human induced mortality. To manage such populations, it is appropriate to consider each of the two islands as separate sub-species. Within the South Island, the populations may be further subdivided into three demographically independent Management Units – the East, West and South Coasts. The South Coast management unit is vulnerable due to its low abundance and isolation and requires further investigation. Population modelling will need to reflect the fact that the local populations within the East and West coast regions share only limited dispersal with immediately adjacent populations and are thus susceptible to fragmentation. These results also show that the population declines of the East Coast South Island and the North Island populations are of recent origin thus implicating fisheries-related mortality as the principal threat to Hector's dolphin. To prevent further decline or fragmentation of South Island populations more stringent control of inshore gillnet fisheries is required. By contrast, current decline of the North Island population may be a result of inbreeding depression. Given the low abundance and rapid decline of the North Island population, it is imperative to evaluate the potential for inbreeding depression while continuing to mitigate all human-related threats.

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3	Pichler, F.B. and Olavarria B, C. (2001) Resolving Chilean dolphin ( <i>Cephalorhynchus eutropia</i> , Gray 1846) synonymy by sequencing DNA extracted from teeth of museum specimens. <i>Revista de Biología Marina y Oceanografía</i> , <b>36</b> , 117-121.	170
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