Molecular ecology of marine mammals

Morten Tange Olsen
Abstract

Marine mammals comprise a paraphyletic group of species whose current abundance and distribution has been greatly shaped by past climate fluctuations and anthropogenic impacts. This thesis describes molecular ecological approaches to answer questions regarding habitat requirements, genetic differentiation, and life-history trade-offs in three species of marine mammals.

The annual sea-ice dynamics of the Arctic may have large effects on the abundance and distribution of Arctic species such as the pagophilic ringed seal (Pusa hispida). Paper I describes and applies a simple molecular method for isolating and characterizing a relatively large set of single nucleotide polymorphisms (SNPs) in the ringed seal. These SNPs have been genotyped in a yet-to-be-analysed dataset which will form the basis in an assessment of the micro-evolutionary effects of annual sea-ice dynamics on ringed seal.

Current management efforts directed towards the North Atlantic fin whale (Balaenoptera physalus) are hampered by an unclear understanding of population structure. Paper II investigates the DNA basis for the high levels of genetic differentiation that have been reported in allozyme studies of the North Atlantic fin whale. We find that additional processes (at the organismal level) may have contributed to shaping the phenotype of the underlying allozyme variation.

Telomeres may potentially serve as markers for determining the chronological and biological age of animals where other means of inference is difficult. Paper III describes the application and evaluation of four qPCR assays for telomere length estimation in humpback whales (Megaptera novaeangliae), finding that reliable telomere length estimates require extensive quality control. Paper IV applies the best performing qPCR assay to test whether telomeres may provide a method for genetic determination of chronological age in whales and concludes that the biological and experimental variation in telomere length estimates is too large to determine age with sufficient resolution. Finally, because telomere length and rate of telomere loss also may be affected by other cellular and organismal processes, such as resource allocation among self-maintenance mechanisms, growth and reproduction, Paper V describes the correlations between individual telomere length and rate of telomere loss, and sex, maturity status and female reproductive output. We found that the costs of reproduction in terms of telomere loss are higher in mature humpback whales than in juveniles; that reproductive costs are higher in males than females; and that differences among females tend to correlate with reproductive output.

Keywords: Marine mammals, molecular ecology, life history, environmental change, phenotypic plasticity, SNPs, telomeres
List of papers

This thesis is based on the following five papers, listed by study species and identified in the text by their Roman numerals:


II. **Olsen MT**, Pampoulie C, Daniëlsdóttir AK, Lidh E, Bérubé M, Víkingsson GA, Palsbøll PJ (submitted) Nucleotide variation at MDH-1 and MPI in North Atlantic fin whales (*Balaenoptera physalus*) indicate that allozyme variation reflects phenotypic plasticity and not population genetic structure. *Molecular Ecology*

III. **Olsen MT**, Bérubé M, Robbins J, Palsbøll PJ (submitted) Empirical evaluation of humpback whale telomere length estimates; quality control and factors causing variability in the singleplex and multiplex qPCR methods. *BMC Genetics*

IV. **Olsen MT**, Robbins J, Bérubé M, Palsbøll PJ (manuscript) Telomeres as proxies for cetacean age and life histories.

V. **Olsen MT**, Robbins J, Bérubé M, Palsbøll PJ (manuscript) Sex-specific costs of reproduction in a long-lived mammal; the humpback whale

The appendix lists six additional papers that were produced in parallel to this thesis.
Acknowledgements

These pages mark the completion of my Ph.D. thesis on the “Molecular Ecology of Marine Mammals” at Stockholm University. I think most PhD candidates start their doctoral project with an anticipation best described by Neil Armstrong when setting foot on the Moon; “One small step for a man, one giant leap for mankind” … at least I did! Soon, however, reality catches up, and one realize that in fact it is the other way around “One giant leap for a man, one small step for mankind”, and that nothing of it would have been possible without the help of many others.

First, I would like to thank my PhD supervisors Per J. Palsbøll and Martine Bérubé for giving me the opportunity to pursue a dream and career based on something as odd albeit fascinating as the molecular ecology of marine mammals. I greatly treasured your help and patience when I got stuck in the mud, and do not know of anyone that could have taught me the virtue of professional expertise and sense of quality and thoroughness as well as you have. It has truly been a very exciting, fun and educative experience!

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1. Introduction

“Among the four-footed animals the seal genus is one of the least accurately described. In the works of most authors one finds a pronounced confusion, of which it is difficult to make sense. The reason is that the seal is a marine animal and rather unapproachable in its natural environment”

Otto Fabricius, 1790

The distribution and abundance of organisms is not static but change over time, as a result of e.g. long-term geological change, seasonal fluctuations, or anthropogenic impacts. Marine mammals comprise a paraphyletic group of species where evolutionary diversification and adaptation in association with the transition from a terrestrial to a marine existence has led to major (often convergent) modifications of their ancestral terrestrial mammal “bauplan” (e.g. morphology, anatomy and physiology), behaviour, and life-history traits (Uhen 2007). Following their entry into the sea, marine mammals have undergone additional radiations into pre-existing and novel niches, arising as geology and climate changed their marine habitat (Kellogg 1928; Davies 1958). As a consequence, extant marine mammals represent a variety of species with very different behaviours and life histories, found in a diverse array of lake, riverine, coastal, and pelagic habitats and in Polar, Temperate and Tropical regions (Pompa, Ehrlich et al. 2011).

Elucidating the processes leading to such radiation and their effects on the distribution and abundance of populations and species, is fundamental for the study of evolution (Darwin 1859). In management and conservation, such understanding is paramount for assessing the relative effects of anthropogenic impacts, and in particular exploitation, thereby aiding in predicting the effects of future anthropogenic impacts. Efforts towards this end include assessing distribution, population structure, abundance, seasonal movements and life history; as well as effects of directed and incidental takes (IWC 2012).

Some key limitations in the study of marine mammals include their generally submerged existence, wide ranges of movements, and often large sizes. Consequently, marine mammals are typically inaccessible to direct observation and handling. The indirect inference methods offered by molecular ecology provide a solution to this problem (Hoelzel 1992), and have been widely used to investigate the relationship between evolution, life-history traits, and environment in marine mammals; as has been the case for many other organismal groups. Molecular ecology applies population and evolutionary genetic theory to molecular genetic data to answer ecological and conservation questions. In particular, molecular ecology has been applied to assessments of population and social structure,

As part of the research underlying this thesis, I have applied molecular ecological approaches to answer questions regarding environmental effects on microevolution, genetic differentiation, age determination, and life-history trade-offs in three species of marine mammals; the ringed seal (*Pusa hispida*), the humpback whale (*Megaptera novaeangliae*), and the fin whale (*Balaenoptera physalus*). The overall objectives were to:

I) Characterize sequence variation in DNA sequences known to contain single nucleotide polymorphisms (SNPs) in the ringed seal (*Paper I*). These SNPs may subsequently be applied to assess the micro-evolutionary relationship between annual sea-ice dynamics in the Arctic and ringed seal population structure, abundance, and life-history. A better understanding of this relationship may aid in predicting the potential effects of the current global warming on the abundance and distribution of Arctic pagophilic marine mammals.

II) Investigate the basis at the DNA level for the high levels of genetic differentiation that have been reported in allozyme studies of the North Atlantic fin whale (*Paper II*). A better understanding of the population structure is important for the conservation and management of North Atlantic fin whales.

III) Develop and evaluate a quantitative polymerase chain reaction (qPCR) assay for telomere length estimation in humpback whales (*Paper III*), in order to test possible correlations between telomere length and chronological age, and potentially develop a qPCR assay for determining the age of free-ranging baleen whales; a fundamental albeit often unknown parameter in the study of marine mammals (*Paper IV*). In addition, since telomere length and rate of telomere loss also may be affected by other cellular and organismal processes, such as resource allocation among self-maintenance mechanisms, growth and reproduction, I also tested correlations between individual telomere length and rate of telomere loss, and sex, maturity status and female reproductive output, to determine whether telomeres may be used as markers of reproductive investments and costs in humpback whales (*Paper V*).

I have organised the thesis in the following manner: first, I give an introduction to marine mammals (*Section 2*), including a definition, their taxonomy, their origin and evolution, and their adaptations to the marine environment. I then proceed to discuss past and future anthropogenic impacts, as well as
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questions and concerns relating to marine mammal conservation and management. Hereafter follows an overview of the field of molecular ecology (*Section 3*), including a brief introduction to the underlying population and evolutionary genetic theory, tissue sampling and DNA extraction methods, traditional and emerging molecular markers and genotyping/sequencing methodologies, as well as traditional and potential future applications of molecular ecology in the study of marine mammals. This general background is followed by three sections (*Sections 4-6*), which specifically relate to the three different projects, I have been involved in during the course of my PhD studies. Each of these sections contain a description of the study species, presents the overall objective(s) of the specific projects and a brief summary of the results, as well as discussion of future directions. To minimize redundancy, the methods applied in each project are presented in the associated manuscripts. I hereafter list my contributions to the various parts of each project (*Section 7*), and conclude the introduction with a brief epilogue (*Section 8*). Hereafter follows a list of references (*Section 9*), as well as an appendix (*Section 10*), listing the additional publications, submitted manuscripts, and conference and workshop presentations, which I have produced during the course of this PhD project. Kindly note that *Sections 1-10* are not expected to provide an in-depth comprehensive review of all these many aspects of my doctoral research, but rather to provide complementary background information. This information should enable an informed reader to understand and evaluate the work presented in the subsequent five manuscripts (*Papers I-V*), which form the basis of this doctoral thesis. Enjoy!
2. Marine mammals

2.1 What is a marine mammal?

Marine mammals comprise a diverse group of species which are characterized by living in and obtaining their food from the sea (Plates 1-4). Contrary to other marine vertebrates such as fish and sharks, the ancestors of marine mammals were terrestrial for 100s of millions of years before gradually entering and adapting to the marine existence (Kellogg 1928; Uhen 2007; Marx and Uhen 2010). Fossil and molecular evidence suggest that such entries happened on at least seven different occasions given rise to the extinct Desmostylia and Thalassocnus spp. (aquatic sloths), as well as the extant monophyletic groups Cetacea (whales, dolphins and porpoises), Pinnipedia (seals, sea lions, and walruses), and Sirenia (dugongs and manatees), as well as the polar bear (Ursus maritimus), the sea otter (Enhydra lutris), and the marine otter (Lontra felina) (Arnason 1974; Arnason 1974; Barnes, Domning et al. 1985; Irwin and Arnason 1994; Shoshani and McKenna 1998; Uhen 2007). For brevity, the remaining chapters focus on the two most diverse groups of extant marine mammals; the cetaceans and pinnipeds, which are the species groups my thesis is based upon.

2.2 Taxonomy

Cetaceans belong to the Cetartiodactyla and are divided into the toothed whales (Odontoceti) and the baleen whales (Mysticeti). There are about 72 extant species of toothed whales, which are divided into 10 families including the Delphinidae (dolphins, killer whales, pilot whales, and some river dolphins), Monodontidae (narwhal and beluga), Phocoenidae (porpoises), Kogiidae (dwarf and pygmy sperm whales), Physeteridae (sperm whales), Ziphiidae (beaked whales), as well as the river dolphin families Platanistidae, Iniidae, Pontoporiidae, and Lipotidae, whose exact phylogenetic relationship is debated (Arnason and Best 1991; Geisler, McGowen et al. 2011; SMM 2011). In contrast to the large diversity within toothed whales, the extant baleen whales only amount to 14 species divided into four families including Balaenidae (right whales and bowhead whale), Neobalaenidae (pygmy right whale), Balaenopteridae (rorquals), and Eschrichtiidae (gray whales) (SMM 2011). All pinnipeds belong to the order Carnivora and include the families Odobenidae (the walrus) with one species, Otariidae (eared seals, including sea lions and fur seals) with 14 species, and the Phocidae (the earless or true seals) with 19 species (Davies 1958; Arnason, Gullberg et al. 2006; Fulton and Strobeck 2010; SMM 2011).
Plate 1: Cetaceans. Top: The killer whale (Orcinus orca) is a cosmopolitan toothed whale, here feeding on fish in northern Norway. Middle: The southern right whale (Eubalaena australis) is a southern Hemisphere baleen whale, here at the breeding area in South Africa. Bottom: The humpback whale (Megaptera novaeangliae) is a cosmopolitan baleen whale, here feeding in the Gulf of Maine, US. Notice the characteristic white pectoral fins. Photos: Morten Tange Olsen.
Plate 2: Pinnipeds. Top: The brown fur seal (Arctocephalus pusillus) is a southern Hemisphere eared seal feeding on fish and breeding in large colonies, here in South Africa. Bottom left to right: The harbour seal (Phoca vitulina) and the bearded seal (Erignathus barbatus) are true seals, but whereas the harbour seal occur in temperate and some Arctic regions across the northern hemisphere the bearded seal is strictly Arctic. Photos: Morten Tange Olsen.

Plate 3: Sireniants. The West Indian manatee (Trichechus manatus) and other sea cows are exceptional among marine mammals in being herbivorous and all extant forms are restricted to temperate coastal regions, swamps, rivers and wetlands, here in Florida. Photos: Morten Tange Olsen, except upper right from www.wikipedia.org
Plate 4 – Polar bear: The polar bear (Ursus maritimus) is an Arctic top predator and characterized as a marine mammal because of its frequent use of the marine environment, here off of Cape Farewell, southern Greenland. Photos: Morten Tange Olsen.
2.3 Origin and evolution

A central driver of marine mammal origin and diversification was the increased primary and secondary biological productivity in the marine realm caused by geological restructuring of the oceans and climate fluctuations (Lipps and Mitchell 1976; Fordyce 1977; Uhen 2007; Marx and Uhen 2010).

The earliest mammal fossils with a cetacean-like bauplan stem from the early Eocene Climatic Optimum (50 million years ago (mya)) (Kellogg 1928), a period characterized by warm, broad and shallow seas with high productivity in coastal regions (Figure 1) (Zachos, Pagani et al. 2001). During the Eocene (56-33 mya), a gradual cooling of the Earth resulted in an increased upwelling of nutrient rich bottom layers leading to a high primary and secondary productivity (Zachos, Pagani et al. 2001), which presumably facilitated the initial radiation of early (Archaic) whales into different ecological niches, starting in the riverine and shallow marine settings before colonizing the open oceans (Uhen 2007). This period also saw the origin of pinnipeds, as suggested by the fossil record (Davies 1958; Fulton and Strobeck 2010).

At the Eocene-Oligocene transition (33 mya), continuous cooling, as well as the opening of the Tasmanian-Antarctica and Drakes’ passages and the resulting establishment of the Antarctic Circumpolar Current, facilitated a dramatic increase in primary and secondary productivity in the waters off Antarctica, New Zealand and Australia (Kennett 1977), presumably enabling the evolution of filter feeding baleen whales and consequently the split between toothed and baleen whales (Lipps and Mitchell 1976; Fordyce 1977; Fordyce 1980; Marx and Uhen 2010).

The fossil record and phylogenetic analyses indicate that most extant baleen and toothed whale families originated during the Oligocene-Miocene transition (23 mya), during which the Archaic whales gradually became extinct (Uhen 2007; Steeman, Høbsgaard et al. 2009). The species diversity observed among modern baleen and toothed whales was likely driven by subsequent niche differentiation generated by climatic fluctuations and the formation of Antarctic ice-sheets as well as sea level variations of up to 200 m, causing modern cetaceans to peak at more than 200 species during the mid-Miocene (15 mya) (Kellogg 1928; Davies 1963; Morlon, Parsons et al. 2011). The origin and diversification of the pinnipeds was to a large extent driven by similar processes and events, but in particular adaptations to the icy Polar Regions at the Oligocene-Miocene transition appear to have been a prominent factor leading to the split of pinnipeds into the true seals and eared seals (incl. walruses) (Davies 1958; Arnason, Gullberg et al. 2006).
The Mid and Late Miocene (15-5 mya) was characterized by geological and climatic changes, including the initial formation of Arctic ice sheets, as well as restructuring of the oceans, such as the closure of the Tethys Seaway between Africa and Europe/Asia (i.e. in the Mediterranean Sea-Persian Gulf region) separating the North Atlantic from the Indian Ocean and a gradual narrowing of the Indo-Pacific Seaway restricting movements between the Indian Ocean and the South Pacific. The relatively high diversity within Delphinidae, Phocoenidae, Ziphiidae, and Balaenopteridae arising during this period is likely a result of these separations between the major oceans; as well as periods of global warming that fragmented upwelling areas of high productivity in the Arctic and Antarctic (Davies 1963; Rosenbaum, Brownell et al. 2000; Pastene, Goto et al. 2007; Steeman, Hebsgaard et al. 2009; Marx and Uhen 2010). In contrast to these four species rich families, all other cetacean families (Monodontidae, Kogiidae, Physeteridae, Platanistidae, Iniidae, Pontoporiidae, Lipotidae, Balaenidae, Neobalaenidae, and Eschrichtiidae), declined to their current lower diversity at a total of approximately 14 species (Morlon, Parsons et al. 2011; SMM 2011). Like modern cetaceans, pinniped species richness increased during the Mid-Miocene climatic fluctuations during which eared seals and walruses appear to have separated (Fulton and Strobeck 2010). Phylogenetic analyses further suggest that northern and southern Hemisphere true seals separated and radiated during the Miocene-Pliocene transition (5 mya) (Fulton and Strobeck 2010).

During the Pliocene and Pleistocene (5 mya – present), several cetacean lineages, and perhaps in particular the Delphinidae, underwent additional radiations presumably due to the closure of the deep Indo-Pacific Seaway, restricting connectivity between the Pacific and Indian Ocean (4 mya), as well as the rise of the Isthmus of Panama, which separated the Atlantic from the Pacific Ocean (3 mya) (Steeman, Hebsgaard et al. 2009). While some diversification within the sea lions and true seals are phylogenetically dated to the separation of the Atlantic and the Pacific Ocean, the main radiation of pinnipeds date to the onset of the northern Hemisphere glaciations (2.5 mya), resulting in several new species and subspecies within the *Phoca-Pusa* complex (Davies 1958; Arnason, Gullberg et al. 2006; Fulton and Strobeck 2010).

As is evident from the above, marine mammals have diversified in response to overall similar processes, but adapted to different habitats; as testified by the current distribution of marine mammal diversity (Pompa, Ehrlich et al. 2011) (Figure 2).
Figure 1 Climatic and geological changes, as well as major events in marine mammal evolution during the past 60 million years. Geological events: I. Opening of Tasmania-Antarctic Passage; II. Opening of Drake Passage; III. Gradual closure of Tethys Seaway; IV. Closure of the deep Indo-Pacific Seaway and rise of the Isthmus of Panama. Marine mammal evolution: A. First Archaic cetaceans; B. First pinnipeds; C. Split between toothed and baleen whales; D. Split between eared and earless seals; E. Radiation of pinnipeds and several cetacean families; F. Radiation of pinnipeds and delphinids. Time periods: Pal. Palaeocene; Eo. Eocene; Ol. Oligocene; Mio. Miocene; Pli. Pliocene; Plt. Pleistocene. Figure modified from www.wikipedia.org
Figure 2 Global distribution of marine mammal species richness. A: Pinnipeds (seals and walruses). B: Mysticetes (baleen whales). C: Odontocetes (toothed whales). Yellow denote areas with low species richness; blue areas with high. Note the strong congregation in certain geographical areas within and among marine mammal lineages. From Pompa and co-authors (2011), with permission.
2.4 Adaptations to the marine environment

Although highly modified to a marine existence, marine mammals have retained many of their original mammalian features and have an overall similar body plan ("bauplan") (e.g. Kellogg 1928). The transition from the terrestrial to the marine environment provided new evolutionary opportunities, but also challenges. The following paragraphs highlight some of the major, often remarkably convergent, adaptations to the marine existence of marine mammals; as also described in detail elsewhere (Kellogg 1928; Davies 1958; Reidenberg 2007; Uhen 2007).

Among marine mammals, cetaceans show the largest anatomical and ecological specializations to a marine existence (Kellogg 1928). Locomotive adaptations in cetaceans include modification of the skeleton for muscle attachment rather than keeping the body upright; flattening and elongation of the forelimbs; loss of hind limbs; a more streamlined body and loss of hair; as well as a more vertically flexible vertebrate column and a horizontal fluke for propulsion (Kellogg 1928; Uhen 2007). In pinnipeds, swimming is facilitated by having short (true seals and fur seals) or no fur (elephant seals, walruses), a horizontally flexible vertebrate column, and modified hind limbs (flippers) for propulsion (Davies 1958; Uhen 2007). Pinniped flippers fan out in a vertical plane when swimming, similar to the fins of fish and sharks, and opposite to the horizontal fluke of cetaceans (and Sirenia). Eared seals (Otariidae) are able to turn their hind flippers forward and walk on all fours, at the cost of swimming speed, but presumably gain of comparatively greater manoeuvrability on land and in water, whereas true earless seals (Phocidae) have lost the terrestrial support of their hind limbs (Davies 1958; Uhen 2007). Most cetaceans and pinnipeds have evolved thickened layers of subcutaneous fat (i.e. blubber) for insulation and energy storage, presumably in response to the higher conductivity of water relative to air, low temperatures and/or a seasonally patchy distribution of food resources (Davies 1958; Uhen 2007). Respiratory adaptations include and increased capacity for exchanging, absorbing and storing oxygen, and, in the cetaceans; substantial modifications of the respiratory tract (Uhen 2007).

Marine foraging and niche specialization also entailed major adaptations, and likely lead to the diversification of the toothed whales and the baleen whales (Lipps and Mitchell 1976; Fordyce 1977; Marx and Uhen 2010). The toothed whales are raptorial echolocators, preying on fish and squid, or other marine mammals. In contrast, baleen whales are filter feeders, using large baleen plates to filter zooplankton (notably krill and copepods) and fish from the water (Fordyce 1980). The main distinctiveness of the baleen whale skull are the elongated jaws that support the baleen, whereas the toothed whale skull, besides having teeth, is characterized by dish-shaped frontal depressions in which the sound-producing and enhancing soft tissues used for echolocation are located (Kellogg 1928; Kellogg 1958). Filter feeding allowed baleen whales to feed more efficiently at lower trophic levels,
which has been hypothesized to facilitate the subsequent evolution of larger baleen and larger bodies (Fordyce 1980). Pinnipeds are also aquatic foragers and, like cetaceans, have adapted to a wide variety of prey sources. Many species feed on fish and squid and have retained much of the original carnivore tooth morphology, whereas e.g. crabeater seals (*Lobodon carcinophagus*) have evolved a unique set of filter-like teeth to prey on krill in the Antarctic (Siniff 1991).

Finally, a significant difference between pinnipeds and cetaceans is that the former group has retained the need for a solid substrate (e.g. land or ice) for resting, moulting and birth. Moreover, in otariids and some phocids, mating occur on land, which in some cases has evolved into polygyny, probably leading to sexual size dimorphism (Bartholomew 1970; Hoelzel, Le Boeuf et al. 1999). In contrast, most aquatic mating phocids have reduced or non-existent sexual dimorphism and represent a wide variety of mating strategies such as territorial mate defence and mate guarding (Van Parijs, Hastie et al. 1999; Boness, Bowen et al. 2006; Krafft, Kovacs et al. 2007).

### 2.5 Anthropogenic impacts

#### 2.5.1 Whaling and sealing

Archaeological excavations suggest that coastal human populations have hunted marine mammals for millennia, likely using their meat for consumption, blubber for fuel/lighting, and skin, bones, teeth, and/or baleen for tools, clothing, scrim, and constructions (Stringer, Finlayson et al. 2008). In contrast to supposed palaeo-human impacts on the terrestrial megafauna (e.g. Lorenzen, Nogues-Bravo et al. 2011), it may be presumed that early hunting of marine mammals mainly affected local populations of nearshore species, although these impacts may have been substantial locally (Hildebrandt and Jones 1992; Porcasi, Jones et al. 2000).

**Large cetaceans**

Starting in the 11th century, Basque whaling represents the first larger scale exploitation of marine mammals for commercial purposes (Clapham, Aguilar et al. 2008). This fishery started in the waters off the Basque region, but subsequently expanded to include the waters off the British Isles, Northern Norway, Iceland, Greenland, Labrador and Newfoundland. Initially, whaling mainly targeted the slow swimming right and bowhead whales which have a very thick layer of blubber, long baleens, and, most importantly, floated when killed, hence being the “right” whales to harvest (Tønnesen and Johnsen 1982). During the 17th–19th century, commercial whaling expanded to the southern Hemisphere and additional species, such as sperm whales (*Physeter macrocephalus*), as North Atlantic
Marine mammals

populations became depleted and the Dutch, British, French, Danes-Norwegians, and Yankees launched their whaling operations (Tønnesen and Johnsen 1982). Towards the end of the period (i.e. the 19th century), whaling operations primarily targeted blubber for lighting and industrial use (e.g. lubrication of machines, manufacturing of soap, textile and tanning) and grew in economic importance with the industrial revolution and human population growth, particularly in North America and Europe, as well as in Australia, New Zealand and South Africa (Tønnesen and Johnsen 1982; Hilt 2007).

Continuous industrialization and human population growth led to increased demands for large quantities of fat and oil. These demands could be met with the technical developments by the Norwegian Sven Foy in the 1860s who introduced the use of steam ships (rather than sail and rowing boats), explosive harpoons fired from a bow-mounted canon (rather than thrown or shot), with a wire attached to the harpoon in order to retrieve the killed whale if it sank (Tønnesen and Johnsen 1982). The technical developments of Foy and others mark the initiation of the modern whaling era which targeted the fast swimming rorquals (e.g. fin, blue, and humpback whales) which until then to some extent had been spared from commercial whaling operations (Reeves and Smith 2002; Reeves, Smith et al. 2002). Traditionally whaling had relied on land stations for processing of harvested whales, which imposed a limit to the operational range of the catcher boats, but the invention of factory ships with rear slipways in the 1920s facilitated wide-ranging pelagic whaling operations and led to further decimation of rorqual and right whale stocks (Tønnesen and Johnsen 1982). The historic abundance and the number of baleen that were harvested are unknown, and subject to much debate (Roman and Palumbi 2003; Baker and Clapham 2004; Jackson, Patenaude et al. 2007). Catch records and accounts provide some information about takes and ship log-books may aid in locating historic breeding and feeding populations. From this, it has been estimated that more than two million baleen whales were killed during the 20th century in the southern Hemisphere alone (Clapham, Aguilar et al. 2008). Hence, the worldwide harvest of large whales may have been in the tens of millions.

A growing awareness that whaling could lead to the depletion of rorquals in manners similar to what had happened to the right whales, lead to gradual regulation of the whaling industry, to ensure the viability of whale stocks for future harvests (i.e. sustainable whaling) (Tønnesen and Johnsen 1982). Initiatives were first local and/or national, but whaling became globally regulated in 1946 by the Washington Convention and forming of the International Whaling Commission (IWC) in 1949 (Tønnesen and Johnsen 1982; IWC 2012). The Convention recognized “that the history of whaling has seen over-fishing of one area after another and of one species of whale after another” and hence “desired to establish a system of international regulation for the whale fisheries to ensure proper and effective conservation and development of whale stocks” (ICRV 1946). The main purpose of the IWC is to review and revise the measures laid down in the Convention, including protecting certain species,
designating whale sanctuaries, and setting quotas for specific species and stocks, as well as funding research and publishing results (IWC 2012).

Despite these initiatives, whaling was continued by some nations and in particular by the former Soviet Union, which undertook large scale illegal whaling from 1947 to 1973 with severe impacts on the recovery of officially protected whale populations (Clapham, Good et al. 2004; Clapham, Mikhalev et al. 2009). The IWC eventually adopted a moratorium on commercial whaling which was put in place in 1982, allowing only catches for aboriginal subsistence and scientific purposes (Tønnessen and Johnsen 1982). At present, aboriginal subsistence hunting occur in e.g. West Greenland, North America (Alaska and Washington State), Canada, the Caribbean, Indonesia, and Philippines (Reeves 2002), while Japan is conducting scientific whaling (Gales, Kasuya et al. 2005; Clapham, Childerhouse et al. 2007). Iceland and Norway also conduct whaling, originally under scientific permit, and since under a veto to the moratorium (IWC 2012). Baleen whales are listed on Appendix I under regulation of the International Union for the Conservation of Nature (IUCN 2012) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2012). Accordingly, whale products may not be exported for commercial purposes. While current whaling operations presumably do not pose a great risk to the specific targeted populations, there is a concern that this type of whaling may provide an outlet and cover of illegal whaling products at markets where prices are high, such as in Japan and Korea (e.g. Baker and Palumbi 1994; Palsbøll, Bérubé et al. 2006). Total catches during the period 1985-2009 amounts to approximately 8,000 whales for aboriginal subsistence (mainly gray whales (Eschrichtius robustus), minke whales (Balaenoptera acutorostrata), and bowhead whales (Balaena mysticetus)); approximately 13,000 whales under scientific permit (majority minke whales (Balaenoptera acutorostrata and Balaenoptera bonaerensis), but also sei whale (Balaenoptera borealis), Bryde’s whale (Balaenoptera edeni) and fin whale (Balaenoptera physalus)); and approximately 20,000 (mainly minke whales) under objection to the moratorium, of which approximately half was taken by Japan and USSR in the mid-1980s, and the other half primarily by Norway since then (IWC 2012).

Small cetaceans and pinnipeds

Small cetaceans (small whales, dolphins and porpoises; i.e. most toothed whales) have also been harvested locally and commercially, and are subject to substantial by-catch, but it is beyond the scope of this dissertation. Many of the eared seals have been targeted commercially and been subject to substantial population declines (Ling 2002), including fur seals (Arctocephalus sp) (Bernardi et al. 1998, Weber et al. 2004, Goldsworthy et al. 2000, Wynen et al. 2000) and elephant seals (Mirounga sp) (Bonnell and Selander 1974, Hoelzel et al. 2001), which at least seasonally occur in large rockeries and hence are easier to harvest in large numbers. Arctic pinnipeds were and continue to be hunted for
commercial or subsistence purposes, as discussed in Section 4.1.8. Also, in regions with high human densities such as northern Europe, pinnipeds have been subject to eradication campaigns by governmental initiative to reduce their impact the fish stocks and fisheries (Olsen, Andersen et al. 2010). Harvest of small cetaceans and pinnipeds is not globally regulated, but the scientific committees of the IWC (small cetaceans) and the North Atlantic Marine Mammal Commission (NAMMCO), regularly address matters regarding their conservation and make management recommendations (IWC 2012). For some species, harvest and trade is regulated by the IUCN and CITES (CITES 2012; IUCN 2012).

2.5.2 Global warming

As should be evident from the above, changing climates since the Eocene have been a major driver of evolution and diversification of marine mammals. In the marine realm, climatic changes affect sea temperature, sea levels, extent and thickness of sea-ice, salinity, acidity, and ocean circulation, which has direct effects on marine mammals through their dependency on specific physical conditions for survival and reproduction (e.g. temperature or substrate), as well as indirect effects through changes in the abundance and distribution of their prey (Simmonds and Eliott 2009). Like other organisms, marine mammals have responded to these changes by range shifts, survival in refugia, changed timing of growth and reproduction, phenotypic plasticity, adaptations, or becoming extinct (e.g. Hewitt 2000; Davis and Shaw 2001; Petit, Aguinagalde et al. 2003; Parmesan 2006; Hoffmann and Sgro 2011).

The current global warming results from anthropogenic processes (i.e. elevated emission of carbon dioxide, methane, and nitrous oxide) and temperatures are rising at an elevated rate causing concerns over the biota's ability to adapt to these changes (Parmesan 2006; IPCC 2007). The potential abrupt and irreversible impacts of global warming may have far reaching social, political, economic and ecological consequences. In current projections, global warming will increase the risk of extinction in 20-30%, or perhaps up to 40-70%, of the Earths flora and fauna (IPCC 2007).

The species at highest risks are likely those with most specific habitat and prey requirements, such as pagophilic marine mammals inhabiting the Arctic and Antarctic (Tynan and DeMaster 1997; Laidre, Stirling et al. 2008; O'Corry-Crowe 2008; Cooper, Budge et al. 2009). The increase in temperature is projected to be highest in the Arctic, and hence the largest environmental effects will likely take place in this region (Vinnikov, Robock et al. 1999; IPCC 2007; Perovich and Richter-Menge 2009). During the past decade, global warming have caused pronounced declines in the extent of summer sea-ice in the Arctic Ocean, which are unmatched in magnitude and duration compared to fluctuations during the previous 1,450 years (Kinnard, Zdanowicz et al. 2011). Changes at community and ecosystem scales have already been reported in Arctic terrestrial systems (Walther, Post et al. 2002; Post, Forchhammer...
et al. 2009), as well as locally in marine mammals. For example, ringed seal recruitment was substantially affected by fluctuations in snow depth and timing of sea ice break up in western Hudson Bay (Ferguson, Stirling et al. 2005) and East Greenland catch records suggest lower abundances of ringed seals (Pusa hispida) and polar bears (Ursus maritimus) in warm years relative to cold (Rosing-Asvid 2006). Ringed seals are among the most numerous and widely distributed of the Arctic pinnipeds and most likely one of the least sensitive Arctic marine mammals (Laidre, Stirling et al. 2008), however other Arctic marine mammals with more specific habitat requirements, such as narwhals (Monodon monocerus), hooded seals (Cystophora cristata), and polar bears may be less capable of adapting (Bluhm and Gradinger 2008; Laidre, Stirling et al. 2008).

In the worst case scenario, global warming and the resulting freshening of the sea may change the thermohaline circulation to the extent that upwelling in high-and mid-latitude areas may be greatly reduced or cease altogether (IPCC 2007). As mentioned above, these upwelling areas are crucial for the marine food web as they bring nutrient-rich deep water to the surface without which the primary and secondary productivity would be substantially reduced (Barber, Lukovich et al. 2008; Greene, Pershing et al. 2008). Long-term changes in upwelling may have consequences for the local marine mammal communities (Bluhm and Gradinger 2008), as well as the distribution and abundance of migratory baleen whale species directly or indirectly relying on the high seasonal productivity in these areas (Benson, Croll et al. 2002; Tynan 2004; Wiedenmann, Cresswell et al. 2011).

2.5.3 Other impacts

In addition to exploitation, humans have and are affecting marine mammals and their ecosystem (Shea and Odell 2008). Global human population growth and increased marked demands is putting high pressures on marine fisheries, causing collapse of fish stocks and interspecific competition for food resources (Jackson, Kirby et al. 2001; Hjermann, Ottersen et al. 2004), as well as increasing incidental by-catches with severe consequences for a wide range of marine mammals. In the 1960s and 1970s, 5-10 million dolphins were killed in the tuna fisheries, and commercial fisheries of Dall’s porpoise (Phocoenoides dalli) alone amounted to tens of thousands of animals annually in the 1980s (Hoelzel 1992). More than half a million marine mammals die annually from by-catch in fishing gear (Read, Drinker et al. 2006), and many more are affected by ship traffic, habitat disturbance, pollution, and other anthropogenic impacts (Kaschner, Tittensor et al. 2011). Increased commerce and associated ship traffic result in mortality due to ship strikes (Laist, Knowlton et al. 2001; Douglas, Calambokidis et al. 2008; Williams and O’Hara 2010) and disturbance from underwater noise pollution (Tyack 2008); which will probably increase with efforts to explore mineral resources and other offshore activities (Frost, Lowry et al. 1999; Edren, Andersen et al. 2010; Campagna, Short et al. 2011). Moreover, habitat destruction may lead to declines in local marine mammal populations (Wolff 2000),
and agricultural nutrient run-off may result in disturbance of coastal ecosystems (Karlson, Rosenberg et al. 2002) and cause harmful algae blooms with effects to marine mammals (Flewelling, Naar et al. 2005).

The marine environment, and the Arctic in particular, acts as a sink for global atmospheric pollutants (Simonich and Hites 1995) which accumulate in the food chain (i.e. biomagnification) and ultimately end up in predatory species at the highest trophic levels (Dietz, Paludan-Müller et al. 1998; Dietz, Riget et al. 2006; Aubail, Teilmann et al. 2011). High contaminant loads may cause tissue damage (Sonne, Dietz et al. 2005), hormone disruption (Routti, Arukwe et al. 2010), reduced fertility (Reijnders 1986), and immunosuppression (Reijnders 1986; Olsson, Karlsson et al. 1994; Sonne, Dietz et al. 2006). Marine mammals may also be exposed to relatively higher background levels of pathogens and diseases due to global warming and other environmental stressors (Burek, Gulland et al. 2008; McAloose and Newton 2009; Van Bressem, Raga et al. 2009). Indeed, it has been proposed that the apparent recent increase in mass mortalities resulting from morbillivirus outbreaks in e.g. European harbour seals (Phoca vitulina) and several other marine mammals may have a substantial anthropogenic component (Kennedy, Kuiken et al. 2000; Di Guardo, Marruchella et al. 2005; Harkonen, Dietz et al. 2006; Beineke, Siebert et al. 2010).

2.6 Questions and concerns

As should be clear from the above, the largest determinants of current marine mammal distributions and abundances are past geological and climate changes, as well as anthropogenic impacts, such as exploitation. Given the continued growth of the human population, anthropogenic impacts are likely to intensify in the future. In isolation, any of these potential impacts may not pose serious threat to marine mammals, but their cumulative effects likely will, as testified by the list of species gone extinct within the past centuries. These include the Caribbean monk seal (Monachus tropicalis), the Atlantic gray whale (Eschrichtius robustus), Steller’s sea cow (Hydrodamalis gigas), and the Japanese sea lion (Zalophus japonicas), and possibly the baiji (Lipotes vexillifer) (Pompa, Ehrlich et al. 2011; IUCN 2012). In contrast, species like the Antarctic fur seal (Arctocephalus gazella) (Hoffman, Grant et al. 2011), the northern elephant seal (Bonnell and Selander 1974), and several baleen whale subpopulations (Clapham, Young et al. 1999), have undergone a remarkable recovery. Still, in many other cases, there are no signs of recovery despite the establishment of protective measures (Clapham, Aguilar et al. 2008), and several species, such as, the vaquita (Phocoena sinus), the baiji (Lipotes vexillifer), and the north Atlantic right whale (Eubalaena glacialis) are at the brink of extinction, and others have unknown conservation status (Pompa, Ehrlich et al. 2011; IUCN 2012). The decline of one species or population may sometimes have indirect effects on other marine mammal species or...
populations. For example, it has been hypothesized that the depletion of fin (Balaenoptera physalus) and blue whale (Balaenoptera musculus) populations are the cause of current unusually high abundances of Antarctic minke whales (Balaenoptera bonaerensis), although genetic data failed to support the idea (Ruegg, Anderson et al. 2010). Also, it has been hypothesized that the recurrent collapse of North Pacific pinniped and sea otter populations result from predation by killer whales (Orcinus orca) which were forced to swift prey when industrial whaling depleted great whale populations (Springer, Estes et al. 2003). Ultimately, loss of marine diversity may have direct impact on ocean ecosystem services through changes in the ocean carbon cycle (Pershing, Christensen et al. 2010), as well as negative effects of biodiversity loss on rates of resource collapse, ecosystem recovery potential, stability, and water quality (Worm, Barbier et al. 2006).

2.7 Summing up

A more thorough understanding of the factors affecting the distribution and abundance of marine mammals is essential for assessing the past, present and future effects of environmental change and anthropogenic impacts on marine mammal diversity. Such inference is important not only because it is a prerequisite for marine mammal conservation and management, but also because marine mammals may be good proxies for detecting changes at other trophic levels, which may be more difficult to monitor (Moore 2008). With data in hand, managers can make informed decisions about appropriate response mechanisms (Lotze and Milewski 2004), such as regulating the global emission of greenhouse gasses and pollutants, and/or the effects of other anthropogenic impacts on sensitive marine mammal populations by lowering hunting and fisheries quotas, reduce by-catch, establishing marine sanctuaries, promoting international cooperation, initiating monitoring efforts, and research funding.
3. Molecular ecology

Molecular ecology is a powerful indirect approach to study otherwise relatively inaccessible marine mammals, combining population and evolutionary genetic theory with molecular genetic techniques to study the evolution and ecology of populations and species. The following provides a brief introduction to the underlying theory and techniques, and point out potential applications within marine mammal science, in particular conservation and management.

3.1 Population and evolutionary genetics

Population and evolutionary genetic theory provide a framework with which to characterize and estimate evolution in terms of changes in allele frequencies within and among individuals and conspecific populations (population genetics; microevolution) and how these changes gradually becomes fixed giving rise to variation among species (evolutionary genetics; macroevolution) (e.g. Hedrick 2005; Allendorf and Luikart 2007). It arose from the integration of genetics and Darwinian evolution under the Modern Synthesis by Huxley (Huxley 1942), drawing from Darwin’s theory of natural selection (Darwin 1859), Mendelian inheritance, theoretical developments of early 20th century population geneticists Fisher, Haldane and Wright (e.g. Haldane 1924; Fisher 1930; Wright 1931; Haldane 1937), and conceptual empirical work by Dobzhansky and others (e.g. Sturtevant and Dobzhansky 1936; Dobzhansky 1948; Dobzhansky 1973), as well as more recent developments (Kimura 1968; Kingman 1982; Hudson 1990).

In brief, the theory resulting from their work describes the four microevolutionary forces: mutation, selection, migration, and genetic drift, and their relative contributions to the tempo-spatial distribution of genetic diversity. Briefly; mutation produce new genetic variation (alleles); selection acts on standing genetic variation and cause change in allele frequencies over generations, typically towards either extreme allele frequencies (directional selection) or intermediate frequencies (stabilizing selection); migration is the movement of genomes between populations (i.e. gene flow), causing homogenization of allele frequencies; and genetic drift denotes the random fluctuations in allele frequencies due to random mating and Mendelian transmission of gene copies between successive generations in finite sized populations, which, in the absence of migration, leads to increasing levels of genetic divergence among populations. The effective population size is the “size of an ideal population that has the same genetic drift as the observed population”, which in rough ecological terms are the number of individuals whose genome are transmitted onto the next generation.
The most fundamental population model in population genetics is the Wright-Fisher population model. A Wright-Fisher population consists of diploid individuals with a constant effective population size, non-overlapping generations, random mating, and equal reproductive success (Fisher 1930; Wright 1931). Under these conditions, the relationship between genetic diversity ($\theta$), the effective population size ($N_e$) and the mutation rate ($\mu$) is $\theta = 4\mu N_e$ (Kingman 1982; Hudson 1990).

Natural populations rarely exist in complete isolation, but are usually connected by some degree of migration. Wright’s Island model (Wright 1940) is the most basic population genetic model which include migration. This model assumes that a population is divided into a number of subpopulations of equal size ($N$) that all exchange individuals with an equal rate ($m$). Under these assumptions the degree of genetic differentiation between subpopulations is given by $F_{ST} = \frac{\sum (1-m)^2}{2N - (2N - 1)(1-m)^2}$. For $m$ below 1%, genetic differentiation can be approximated by $F_{ST} \approx 1/(4Nm+1)$, where $F_{ST}$ is the degree of genetic differentiation among populations and $Nm$ the number of migrants per population (Wright 1940; Waples 1998). It follows that low population size and migration rates will increase the effect of genetic drift on population allele frequencies and result in genetic differentiation among populations. Modifications of the Island model accounts for migration between neighbouring populations (Stepping-Stone model) (Wright 1943) and isolation by distance (Slatkin 1991; Rousset 1997).

Population genetic theory has served as the basis upon which inferences in molecular evolution and evolutionary ecology are drawn (e.g. Lande 1988; Pool, Hellmann et al. 2010). However, as should be evident from the few examples above, these inference rely upon many assumptions that rarely are valid for natural populations (e.g. Whitlock and McCauley 1999). Thus, it is important to keep in mind that population genetic theory can be a powerful tool for understanding evolutionary and demographic processes, but naive or inappropriate use can lead to misinterpretations and biased conclusions, as discussed in Section 3.4.

3.2 Sampling and DNA extraction

Studies of the molecular ecology of natural populations require that individuals and their genomes can be sampled. Early studies of marine mammal molecular ecology relied on samples from stranded animals, whaling operations, incidental takes (by-catch), or live capture (Hoelzel 1992), which besides being lethal or stressful may be susceptible to sampling bias (Bilgmann, Möller et al. 2011). The introduction of biopsy techniques for remote sampling of skin tissue from free-ranging animals (Winn, Bischoff et al. 1973; Aguilar and Nadal 1984; Lambertsen 1987; Palsbøll, Larsen et al. 1991) overcame these limitations and greatly facilitated our understanding
of marine mammal evolution and ecology. Moreover, because most biological material contains DNA, a wealth of alternative methods to sample and extract DNA has emerged, making possible the analysis of DNA from such diverse sources as commercial meat products (Baker and Palumbi 1994), faeces (Reed, Tollit et al. 1997); sloughed skin (Clapham, Palsbøll et al. 1993; Milinkovitch and Dunn 1994); baleen, teeth, bones, and skin from museum collections and archaeological sites (Pääbo 1989; Morin, Hedrick et al. 2007; Wandeler, Hoeck et al. 2007); cetacean blow (Frère, Krzyszczyk et al. 2010); and directly from the aquatic environment (Salling, Thomsen et al. 2011; Thomsen, Kielgast et al. 2011). In combination with the appropriate molecular markers, these sampling and DNA extraction techniques may allow for obtaining a wealth of biological information from contemporary and historic/ancient samples, including species identity (Arnason, Gullberg et al. 1993), individual identity (Palsbøll, Allen et al. 1997), sex (Winn, Bischoff et al. 1973; Bérubé and Palsbøll 1996), diet (Dunshea 2009), and/or viral and bacterial community (Cloeckaert, Verger et al. 2001), as well as the demographic, evolutionary and ecological information that be inferred using population and evolutionary genetic approaches of genes and genomes by use of molecular markers.

3.3 Molecular markers

Detailed accounts on the history and utility of biochemical and genetic markers are numerous (Amos and Hoelzel 1992; Brumfield, Beerli et al. 2003; Zhang and Hewitt 2003; Ellegren 2004; Schlotterer 2004). Over time, several different classes of markers have been characterized and applied such as allozymes, RFLPs, RAPDs, AFLPs, minisatellites, microsatellites, and SNPs, as well as mitochondrial and nucleotide sequences. The main differences between markers relate to them being either biochemical or nucleotides (i.e. DNA or RNA), their mode of mutation (infinite alleles model (Kimura and Crow 1964), infinite sites model (Kimura 1969) or stepwise mutation model (Ohta and Kimura 1973; Kimura and Ohta 1978)), and inheritance (maternal vs. autosomal), as well as cost-efficiency, technical and analytical issues, and potential for automation (Schlotterer 2004). The following provides a brief overview of the markers used during the course of this PhD project, and some of their previous applications to marine mammals.

3.3.1 Allozymes

Allozymes were the first molecular markers for large-scale population analysis, facilitating the shift from a mostly theoretical towards a more empirically dominated field (Hubby and Lewontin 1966; Lewontin and Hubby 1966). Allozymes are allelic variants of enzymes which can be distinguished according to size and charge through gel electrophoresis, allowing to visually examine patterns of diversity within and among populations (Sick 1961). Early applications of allozyme analyses in
marine mammals include Bonnell and Selander’s (1974) now classic assessment of allozyme variation in northern elephant seals (Mirounga angustirostris); a study that simultaneously was among the first to discuss the potentially adverse effects of overexploitation on levels of genetic diversity. The availability of samples from whaling and sealing facilitated a large body of allozyme studies in cetaceans and pinnipeds (e.g. Simonsen, Allendorf et al. 1982; Simonsen, Kapel et al. 1982; Shimura and Numachi 1987; Wada 1988; Wada and Numachi 1991). The main attractiveness of allozymes are that they allow for cheap screening of a large amount of samples and loci (Schlotterer 2004). Limitations include their insensitivity to synonymous mutations and low genetic diversity compared to many other genetic markers, as well as uncertainties regarding the molecular mechanisms giving rise to observed electromorphs. For example, the two allozyme loci MDH-1 and MPI are more or less monomorphic across a range of 15,500 individuals representing 18 different species of toothed and baleen whales (Paper II).

3.3.2 Microsatellites

In the mid-1980s, the field took another big step forward with the introduction of the polymerase chain reaction (PCR), which allowed for in vitro amplification of any genomic region in many individuals based on just small amounts of DNA (Mullis and Faloona 1987). Microsatellites were among the first markers to take full advantage of the PCR technology (Schlotterer 2004), beginning with the isolation and characterization of microsatellites in cetaceans (Tautz 1989; Schlotterer, Amos et al. 1991). Microsatellite loci are abundant throughout the genome (Lander, Linton et al. 2001). They consist of short tandem repeats (1-6 base pair (bp) long), which are added or lost by single-strand slippage during DNA-replication (Tautz 1989). The generally high rates of slippage (mutation rates at $10^{-3}$ - $10^{-5}$/locus/generation) (Ellegren 2004) results in high levels of polymorphism. These characteristics have made microsatellites one of the most commonly employed genetic markers in molecular ecology. In marine mammals, some notable applications include the assessment of social structure, paternity and kinship in pods of toothed whales (e.g. pilot whales (Globicephala melas) (Amos, Schlotterer et al. 1993) and sperm whales (Physeter macrocephalus) (Richard, Dillon et al. 1996), population structure and gene flow of grey seals (Halichoerus grypus) (Allen, Amos et al. 1995), fin whales (Balaenoptera physalus) (Bérubé, Aguilar et al. 1998), harbour seals (Phoca vitulina) (Goodman 1998), and polar bears (Paetkau, Calvert et al. 1995), as well as individual identification and tagging of humpback whales (Palsbøll, Allen et al. 1997). The boom in applications of molecular methods for the study of natural populations was further facilitated by and promoted a continuous development of methods for microsatellite isolation (e.g. Zane, Bargelloni et al. 2002), as well as design of interspecific primers (e.g. Gemmell, Allen et al. 1997; Palsbøll, Bérubé et al. 1997). However, microsatellites are not without their limitations and have issues when it comes to their complex mutation model (Ellegren 2004) and automation of allele calling (Brumfield, Beerli et al. 2003; Morin,
Luikart et al. (2004). Microsatellite genotyping are subject to errors caused by e.g. small allele dominance, stuttering, null alleles, and misinterpretation of banding patterns (Hoffman and Amos 2005), although the contribution from these factors may be substantially reduced by including genotyping controls and/or subsequent data quality control (e.g. Pompanon, Bonin et al. 2005).

3.3.3 Single nucleotide polymorphisms

Single nucleotide polymorphisms (SNPs) have emerged as a viable alternative to microsatellites in the study of non-model species (e.g. Paper I). The major advantages of SNPs compared to microsatellites include; a substantial body of current population genetic theory is based on the presumed mutation model; the high abundance of SNPs in the genome; and the high potential for automation and cross-study comparisons (Schlotterer 2004). SNPs are presumed to evolve according to the infinite sites model (i.e. mutations never occur in the same site twice) (Kimura 1969), have a low mutation rate ($10^{-8}$ per generation), and are biallelic (mostly; but not always because of deviations from the infinite sites model), resulting in a lower information content at SNP loci compared to microsatellite loci. Consequently, more SNPs are needed to achieve comparable genetic resolution and statistical power (e.g. Coates, Sumerford et al. 2009; Morin, Martien et al. 2009; Glover, Hansen et al. 2010), which in turn requires bioinformatic pipelines for data handling and analysis (Nickerson, Tobe et al. 1997; Ewing and Green 1998; Ewing, Hillier et al. 1998; Gordon, Abajian et al. 1998; Morcillo-Suarez, Alegre et al. 2008). The wide adoption of SNPs in the study of non-model species was initially hampered by the issues relating to SNP discovery (Brumfield, Beerli et al. 2003; Morin, Luikart et al. 2004), as well as concerns about the influence of ascertainment bias in the subsequent population genetic inference (Nielsen, Stewart et al. 2000; Wakeley, Nielsen et al. 2001). However these have largely been overcome by novel approaches for SNP discovery and genotyping that utilize massively parallel sequencing technologies (e.g. Seeb, Carvalho et al. 2011), such as RAD-tags (Hohenlohe, Bassham et al. 2010), exome capture (Ng, Turner et al. 2009), and expressed sequence tags (ESTs) (Adams, Kelley et al. 1991) which have recently been used for SNP discovery in Antarctic fur seal (Arctocephalus gazelle) (Hoffman 2011) and North Atlantic right whale (Eubalaena glacialis) (Ierardi, Mancia et al. 2009). Paper I presents some additional aspects of SNPs discovery, genotyping and analysis.

3.3.4 Sequence data

The highest and least biased level of resolution is achieved by determining the nucleotide sequence of a given genomic region (Schlotterer 2004). Sequence data for evolutionary and ecological inference predates the PCR era, but like other DNA based markers have risen in utility and popularity following
the introduction of the PCR. Nucleotide sequence data can be extracted from the mitochondrion or the nucleus. Mitochondrial DNA (mtDNA) is maternally and clonally inherited in mammals, which results in their effective population size being one-fourth the size of autosomal loci (Hedrick 2005). Mitochondrial DNA evolves 5-10 fold faster than most single copy nuclear genes (except for e.g. the MHC) (Brown, George et al. 1979; Wilson, Cann et al. 1985), but slower than microsatellites (Ellegren 2004). Most mtDNA applications in marine mammals are based on the hyper-variable control region and relate to inference of population structure, demographic history, phylogeography, and phylogeny. Early applications in marine mammal science include a worldwide assessment of genetic structuring of humpback whale (Baker, Perry et al. 1993; Palsbøll, Clapham et al. 1995) and harbour seal mtDNA haplotypes (Stanley, Casey et al. 1996), as well as species identification of (legally or illegally) hunted whales sold at Japanese meat markets (Baker and Palumbi 1994). Moreover, because mtDNA is more abundant than nuclear DNA, and hence easier to extract in sufficient quantity and quality, mtDNA has found much use in studies where DNA is extracted from degraded samples (e.g. historic or ancient) (e.g. Gilbert, Bandelt et al. 2005). A limitation associated with the use of mtDNA is that it is maternally inherited, which may pose a problem when inferring population structuring in species with sex-biased dispersal or strong social organisation (Palumbi and Baker 1994), such as many marine mammals. Other limitations are that the mitogenome constitutes a single locus and that inference based on a fragment of the mitogenome (e.g. the control region) is more susceptible to the stochasticity of genetic drift, compared to studies where multiple (nuclear) loci are analysed (Palumbi and Baker 1994). For example, it has recently been indicated that the choice of mitochondrial region affected the precision and/or accuracy of the inferred topologies, mutation rates, and divergence time estimates in toothed whales (Duchene, Archer et al. 2011). This latter bias can be avoided by sequencing the entire mitogenome (Arnason, Gullberg et al. 2006; Morin, Archer et al. 2010; Foote, Morin et al. 2011; Vilstrup, Ho et al. 2011), although it does not solve the single locus problem.

Nuclear DNA is biparentally inherited and recombines (with some exceptions for the sex-chromosomes), and typically evolves at 5-10 fold lower rates than e.g. the mtDNA as noted above. Hence, obtaining sufficient genetic resolution and statistical power usually require sequencing of several loci, which until recently was time-consuming and expensive, particularly in low diversity species such as many baleen whales (Schlotterer, Amos et al. 1991; Jackson, Baker et al. 2009) (e.g. Paper II). However, in recent years, the proliferation of massively parallel sequencing methods, typically referred to as next-generation sequencing (NGS) techniques (e.g. Shendure and Ji 2008; Metzker 2010; Tautz, Ellegren et al. 2010; Glenn 2011) have made time and cost-efficient genome-wide sequencing possible.
3.3.5 Next-generation sequencing

The recent advent of NGS technologies, along with continued development of analytical frameworks and increased in computational power, have brought forth seminal studies of human demographics and evolution (Krause, Fu et al. 2010; Rasmussen, Li et al. 2010; Rasmussen, Guo et al. 2011), and a wide range of new applications, which were unthinkable just a decade ago (Altshuler, Durbin et al. 2010; Green, Guyer et al. 2011). The major advantage of NGS is the ability to generate massive amounts of sequence data at low costs. For example, it took several research groups several years at a cost of hundreds of millions of US dollars to sequence the first human genome with shotgun Sanger sequencing (Sanger, Nicklen et al. 1977; Lander, Linton et al. 2001; Venter, Adams et al. 2001); as of January 2012, Illumina Inc. is offering to sequence and assemble any human genome in 90 days for 5,000 US dollars (Illumina 2012). Several NGS platforms are now available, differing with regard to sequencing strategies and their strengths and weaknesses such as costs, error rate, efficiency, read lengths, and applications (Shendure and Ji 2008; Metzker 2010; Glenn 2011). Some of these applications are described in Section 3.4.

Currently, the nuclear genomes of e.g. the killer whale (Orcinus orca), bottlenose dolphin (Tursiops truncatus), humpback whale (Megaptera novaeangliae), narwhal (Monodon monoceros), Weddell seal (Leptonychotes weddellii), and other marine mammals are in the process of being sequenced (Genome 10K Community of Scientists 2009; MMG 2011). The genome sequence of a species provides basic information on genome structure and genetic diversity, allows for discovery of microsatellites and SNPs, and inform about the location of coding loci and their genetic variation, thus providing a basis for further investigations. Indeed, genome sequencing is a major first step, but it is the NGS technologies’ potential for detailed population and community scale assessments of neutral and adaptive genetic diversity (e.g. Pool, Hellmann et al. 2010), which holds the greatest promise for the field of marine mammal molecular ecology and evolution. Given the recent technological developments, it will soon be practically and economically feasible to generate hundreds of individual genomes, implying that the limiting factor to future molecular ecology applications will be the ability to ask sound biological questions, handle the data and interpret the results, rather than the data generation itself (McPherson 2009; Pool, Hellmann et al. 2010; Tautz, Ellegren et al. 2010).

3.3.6 Telomeres

Telomeres are increasingly being used as markers of individual human lifestyle and fitness (Blasco 2005), and are now slowly emerging for the study of animal populations as well (Monaghan and Haussmann 2006; Dunshea, Duffield et al. 2011). The telomeres of vertebrate genomes consist of
tandem repeated TTAGGG sequences at the end of chromosomes (Meyne, Ratliff et al. 1989). Telomeres play a key role in maintaining chromosomal integrity and are crucial for normal cell function (Blackburn and Szostak 1984). They tend to shorten with age as a function of different cellular and organismal processes (Greider and Blackburn 1985; Allsopp, Vaziri et al. 1992; Richter and Zglinicki 2007). The observation that telomeres shorten with age sparked an interest in using telomeres for genetic age determination of non-model animal species where such parameters are difficult to determine (Nakagawa, Gemmell et al. 2004; Monaghan and Haussmann 2006; Dunshea, Duffield et al. 2011). Age is a fundamental and important parameter for assessing life-history, demographic history, and population ecology, as discussed in more detail in Papers III-V. These papers also include a more thorough description of telomere theory and methodology.

3.4 Opportunities and limitations

As previously stated, molecular ecology has a wide range of applications in the study of natural populations, including assessment of phylogeny and phylogeography; population structure, gene flow and hybridization; paternity, social structure, kinship; fitness and inbreeding; demographic history and population size (reviewed in e.g. Excoffier and Heckel 2006).

3.4.1 Population size, differentiation and management units

Given the severity of anthropogenic exploitation and concerns about future effects of global warming, many molecular ecological applications to the study of marine mammals have a conservation-management-angle, typically focusing on estimating pre-exploitation population sizes and/or contemporary genetic differentiation and migration rates. A common approach to estimating these parameters is rearranging the Wright-Fisher Population model (θ = 4μNe) to estimate effective population size, and/or Wright’s Island model (Fst ≈ 1/(4Nm+1)) to estimate migration rate or number of migrants per generation (Fisher 1930; Wright 1931; Wright 1940). One key assumption of most population genetic inference methods is the notion that populations are in mutation-drift-migration equilibrium. Marine mammal populations (as probably is the case for all natural populations) have likely fluctuated substantially in abundance and distribution as a consequence of climate and geological changes, and many of those population residing or seasonally visiting Temperate and Polar regions probably did not do so until after the last glaciation 10,000 years ago (Stanley, Casey et al. 1996; Palsboll, Heide-Jørgensen et al. 1997; Tolley and Rosel 2006). Hence most natural populations are likely not in mutation-drift-migration-equilibrium, and population size and migration estimates are consequently unlikely to reflect recent or contemporary patterns. For example, low levels of genetic differentiation, as observed in many marine mammals, are often taken to reflect current ongoing
migration, but might as well reflect historic migration (which may have subsequently ceased) (e.g. Landguth, Cushman et al. 2010). Another problem, relating specifically to population differentiation, is that with sufficient highly polymorphic molecular markers, statistically significant $F_{ST}$-values can be detected even at very low levels of genetic differentiation (Waples 1998). Finally, in a conservation and management context, significant levels of genetic differentiation are often used as baselines for delineation of management units (Moritz 1994). However, for the reasons listed above such a criterion may not be appropriate to identify contemporary biological entities (Waples 1998). In addition, populations of management and conservation concern are typically more sensitive to demographic stochasticity than genetic stochasticity (Lande 1988). Hence, rather than focusing on genetic independence, management units should reflect entities of demographic independence, that is, populations where growth is determined by local birth and mortality rates (e.g. Waples 1998; Hedrick 1999; Waples and Gaggiotti 2006; Palsbøll, Bérubé et al. 2007). These three issues illustrate some of the limitations associated with applying genetic (evolutionary) data to contemporary ecological questions. However, this does not imply that population genetics and molecular ecology is of no use; only that care should be taken to apply the most appropriate tools and evaluate the results in a biological context, before jumping to hasty conclusions.

In assessments of population structure, $F_{ST}$ may still be a valid measure of genetic differentiation, as long as it is not translated into an estimate of number of migrants (Whitlock and McCauley 1999), and focus is kept on levels of genetic differentiation rather than whether or not these are statistical significant. In recent years, clustering methods such as STRUCTURE (Pritchard, Stephens et al. 2000; Hubisz, Falush et al. 2009) and GENELAND (Guillot, Estoup et al. 2005) have emerged as valuable complements to traditional $F$-statistics in defining population structure, and the former can also be used to identify migrants. In highly migratory marine mammals and/or those with recent population expansions, kinship-based methods represent a good alternative used for estimating contemporary population structure and migration rates based on the spatial distribution of parent-offspring dyads when the sampling effort is sufficient (Peery, Beissinger et al. 2008; Okland, Haaland et al. 2010; Palsbøll, Zachariah Peery et al. 2010; Skaug, Bérubé et al. 2010).

Less biased estimates of effective population size may be obtained with LDNe (Waples and Do 2008), provided that migration rates are below 5-10% (Waples and England 2011), which may be the case for some philopatric marine mammals (e.g. coastal dolphin populations, some pinnipeds). Several coalescent-based approaches such as IM (Hey and Nielsen 2007) incorporate population structure and changes in population size over time, but may not apply to recent time-scales. These approaches also require substantial computational power for large data sets; a requirement that may be alleviated by using an Approximate Bayesian Computation (ABC) framework (Cornuet, Santos et al. 2008). Also, the ability to obtain DNA from historic or ancient sources allows for the assessment of past levels of
3.4.2 Site fidelity and adaptive variation

Site fidelity to specific feeding and/or breeding areas is a commonly observed phenomenon in both pinnipeds (e.g. Karlsson, Hiby et al. 2005; Hoffman, Trathan et al. 2006; Cameron, Siniff et al. 2007; Campbell, Gales et al. 2008; Wolf, Harrod et al. 2008; Kelly, Badajos et al. 2010) and cetaceans (e.g. Clapham and Seipt 1991; Craig and Herman 1997; Rosenbaum, Pomilla et al. 2009; Valenzuela, Sironi et al. 2009; Herman, Pack et al. 2011). Yet, little is known about the factors that may drive such fidelity. One potential explanation relates to familiarity with an area and its resources and the risks associated with dispersing to other areas (Greenwood 1980), and has been speculated to involve matrilineal transmitted “cultural memory” of specific feeding and breeding sites (Whitehead 1998; Clapham, Aguilar et al. 2008; Valenzuela, Sironi et al. 2009). An alternative and perhaps more likely explanation is local adaptation to the specific environmental conditions at a particular area.

Elucidating the factors conferring local adaption is important in predicting the effects of environmental changes and anthropogenic disturbances, and a fundamental aspect of understanding evolutionary biology. Regrettably, local adaptation and the underlying “genes that matter” is notoriously difficult to detect in natural populations (Storz and Wheat 2010). Cetaceans with morphologically distinct coastal and offshore populations or prey specialization both within and between climatic regions as observed for some dolphins (Douglas, Schnell et al. 1984; Hoelzel, Potter et al. 1998; Rossbach and Herzing 1999) and killer whale ecotypes (Hoelzel and Dover 1991; Foote, Newton et al. 2009; Morin, Archer et al. 2010), may be a good starting point for detecting local adaptation in marine mammals. However, studies in other animals suggest that even in the few cases where individuals of distinct populations are morphological different (Linnen, Kingsley et al. 2009), or their respective habitats harbour clear environmental differences, such as salinity (Bekkevold, Aandre et al. 2005; Whitehead, Roach et al. 2011), altitude (Storz, Runck et al. 2009; Scott, Schulte et al. 2011), or temperature (Fields and Somero 1998), finding and characterizing the molecular basis of local adaptation is not an easy task; let alone determining its effect on fitness (Storz and Wheat 2010; Barrett and Hoekstra 2011). Traditional methods for detecting (candidate) genes under selection include estimating Tajima’s $D$ based on the ratio between number of segregating sites and number of alleles (Tajima 1989), performing the McDonald-Kreitman test based on the ratio between fixed and polymorphic nonsynonymous or synonymous mutations (McDonald and Kreitman 1991), or $F_{ST}$-outlier test (Beaumont and Balding 2004) in which loci showing significantly more or less genetic differentiation are taken for being under divergent or stabilizing selection, respectively (e.g. Paper II).
One of the most common uses of NGS is screening large number of genomes to identify potential genes under selection. For example, NGS have been used to generate expressed sequence tags (ESTs) (Adams, Kelley et al. 1991) in cetaceans and pinnipeds (Ierardi, Mancia et al. 2009; Hoffman 2011), and these ESTs may subsequently be screened for candidate genes under selection. Also, Hohenlohe and co-authors (2010) used RAD-tags to isolate 45,000 SNPs in 100s of sticklebacks, and subsequently estimates of $F_{ST}$ and Tajima’s $D$ in these SNPs to identify potential adaptive differences between freshwater and marine populations. These approaches may add much to our understanding of the environmental factors that affect marine mammal abundance and distribution, the genes that confer local adaptation, and a tool to genetic monitor potential changes resulting from anthropogenic impacts (Schwartz, Luikart et al. 2007).

### 3.5 Summing up

Technical and analytical approaches are continuously being developed, allowing for inference at both evolutionary and ecological time-scales under increasingly more complex population models. Still, it is important to acknowledge that any inference is based on a simplification of reality, which may potentially bias the outcome if the assumptions are incorrect (Excoffier and Heckel 2006).
4. SNPs, Arctic ringed seals and climate change

4.1 The Arctic ringed seal

4.1.1 Taxonomy and phylogeny

The ringed seal (*Pusa hispida*) is a relatively small northern Hemisphere true seal (Phocidae) (Figure 3). It is closely related to the Baikal seal (*Pusa sibirica*) and Caspian seal (*Pusa caspica*) from which it diverged during the Pliocene and Pleistocene (Arnason, Gullberg et al. 2006; Palo and Vainola 2006; Fulton and Strobeck 2010). The taxonomic status of *Pusa* is under debate and the three species are sometimes assigned to the subgenus *Phoca* (Arnason, Gullberg et al. 2006). Ringed seal systematics are characterized by several unresolved issues, but the species is commonly divided into at least five distinct subspecies, which are believed to have become isolated after the Last Glacial Maximum as ringed seal populations tracked the retreating sea-ice and ice sheets northward from their more southern refugia (Reeves 1998; Palo, Makinen et al. 2001; Kelly, Bentson et al. 2010). These subspecies include the widely distributed Arctic ringed seal (*Pusa hispida hispida*); the relatively isolated Okhotsk ringed seal (*Pusa hispida ochotensis*) and Baltic ringed seal (*Pusa hispida botnica*); and the freshwater land-locked Ladoga ringed seal (*Pusa hispida ladogensis*) and Saimaa ringed seal (*Pusa hispida saimensis*). The following sections focus on the Arctic ringed seal (*Pusa hispida hispida*).

4.1.2 Species description

Ringed seals are relatively small compared to other phocids, measuring 1-1.5 meter in length and weighing approximately 70 kg (Krafft, Kovacs et al. 2006). Pups are born with a natal coat (lanugo) and cannot swim until this coat is shed after 4-6 weeks (Reeves 1998). Mature males and females have approximately equal body sizes and pelage colouration (Reeves 1998). Size and pelage colourization (i.e. light vs. dark) may differ among regions and habitat types, and different morphs may differ in foraging behaviour; observations that have been interpreted by Inuit and scientists as the existence of different ringed seal ecotypes (Smith and Hammill 1981; Finley, Miller et al. 1983; Furgal, Innes et al. 2002).
4.1.3 Distribution, habitat and abundance

The Arctic ringed seal subspecies, has a circumpolar distribution and is adapted to take advantage of the snow and sea-ice habitat for reproduction and overwintering (Reeves 1998). Ideal ringed seal habitat is characterized by stable winter and spring sea-ice with dense snow cover, which breaks up during the summer, facilitating high biological productivity (e.g. Freitas, Kovacs et al. 2008). Such habitat is typically found in Arctic fjords and bays with land-fast winter ice and glacial fronts (Smith and Stirling 1975), however large densities of ringed seals have also been reported in offshore areas with relatively stable pack ice, such as the edge of the land-fast ice or in polynyas (Finley, Miller et al. 1983). Ringed seals are the most abundant seal species in the Arctic. Current population size estimates are associated with much uncertainty, but are believed to be on the order of 6-7 million ringed seals (Reeves 1998).

4.1.4 Movements and population structure

Ringed seals were among the first Arctic seals for which detailed movement data was obtained using satellite radio tracking (Heide-Jørgensen, Stewart et al. 1992). Subsequently a long series of studies have followed, revealing complex movement behaviours varying with region, season and individual maturity status. In regions where land-fast ice forms during the winter, ringed seals overwinter in prime habitat in fjords and bays where they maintain breeding holes and form subnivean lairs (i.e. small caves) in the snow. Ringed seals have also been observed to overwinter in the pack-ice of polynya such as the North Water in northern Baffin Bay (Teilmann, Born et al. 1999; Born, Teilmann et al. 2004) or at the edge of the land-fast ice (Finley, Miller et al. 1983; Freitas, Kovacs et al. 2008). Both immature and mature animals have relatively small home ranges during the winter, but these ranges increase substantially during the open water foraging period (Born, Teilmann et al. 2004; Kelly, Badajos et al. 2010). Mature ringed seals exhibit strong inter-annual site fidelity to wintering sites, but may undertake long-distance foraging trips during the spring and summer (Gjertz, Kovacs et al. 2000). It has been hypothesized that such site fidelity increases individual fitness (reproductive output) facilitated by an acquired habitat familiarity, as well as mate availability, and predator avoidance (Emlen and Oring 1977; Greenwood 1980). Immature animals tend to be more mobile than mature animals and will more often displace to the pack-ice during the winter, as well as undertake long-distance movements during the spring and the summer (Born, Teilmann et al. 2004; Freitas, Kovacs et al. 2008; Kelly, Badajos et al. 2010). It is currently unclear if the site fidelity of mature seals reflects natal philopatry or whether the long-distance movements of immature animals will result in permanent spatial displacement from the natal breeding sites (Kelly, Badajos et al. 2010). The reported lack of geographic variation in vocal repertoire points to the latter; however it has also been proposed
to be an adaptive response to polar bear predation (Stirling 1973; Stirling and Thomas 2003). Similarly, levels of genetic differentiation among ringed seals are generally low (Davis, Stirling et al. 2008), however while this may suggest low levels of natal philopatry, it may also result from large effective population sizes and population substructuring being of a too recent origin to be detected by means of conventional population genetic methods, as discussed above.

Figure 3 The Arctic ringed seal has a circumpolar global distribution and is closely associated with ice. Photo: Aqqalu Rosing-Asvid with permission. Maps: modified from www.wikipedia.org
4.1.5 Life-history

Ringed seals become mature at the age of 5-7 years, reproduce at age 7-8 years, and may live for up to 40 years (Reeves 1998; Holst, Stirling et al. 1999), although there is considerable spatial and temporal variation in these estimates (Stirling 2005; Krafft, Kovacs et al. 2006). The life cycle of ringed seals is intimately tied with annual sea ice dynamics and snow cover (Laidre, Stirling et al. 2008). Mature ringed seals overwinter in the water and in subnivean lairs on stable ice. Both males and females make subnivean lairs, presumably to avoid predation, as well as for females to give birth to and nurse pups (Smith and Stirling 1975). Females give birth in April-May and lactation lasts for 5-7 weeks during which the pup puts on considerable weight. Mating takes place underwater after the pup is weaned, but implantation is delayed for 3-4 months and hence foetal development spans 7-8 months (Reeves 1998; Stirling and Thomas 2003). Ringed seals moult as the ice breaks up in May-July, which is followed by an intense foraging period in July-October until the winter ice forms in early November.

The mating system of the ringed seal is to a large extent unknown due to the difficulties with conducting behavioural studies in the Arctic during the breeding season, but is assumed to be polygynous (Yurkowski, Chambellant et al. 2011). Alternative mating tactics can occur and may vary individually and regionally as observed in e.g. hooded seals (*Cystophora cristata*) (Kovacs 1990) and harbour seals (*Phoca vitulina*) (Coltman, Bowen et al. 1999; Van Parijs, Hastie et al. 1999; Van Parijs, Hastie et al. 2000). Mature animals are not gregarious, but both sexes may have overlapping home ranges and share breathing holes (Stirling and Thomas 2003; Kelly, Badajos et al. 2010). Mature males make underwater vocalizations during the breeding period, and also secrete a malodorous substance, which may function in attracting females and/or establishing dominance ranks among males (Stirling and Thomas 2003) and scent marking of female breeding holes and lairs (Hardy, Roff et al. 1991; Ryg, Solberg et al. 1992), respectively. Prime breeding areas are devoid of immature individuals and mature males are often observed with bite wounds. All in all these observations suggest a mating system best described as mate guarding and/or territoriality in which males guard primary female breathing holes or territories and await mating opportunities (Krafft, Kovacs et al. 2007; Kelly, Badajos et al. 2010).

4.1.6 Foraging and diet

Ringed seals exhibit highly flexible foraging strategies and diet with regional and seasonal variability depending on biological productivity (Teilmann, Born et al. 1999; Gjertz, Kovacs et al. 2000; Born, Teilmann et al. 2004). Some animals preferentially forage at glacial fronts in fjords and bays with relatively shallow waters, whereas others seek deeper waters at the offshore ice edge or in polynias
Ringed seal SNPs

(Weslawski, Ryg et al. 1994; Freitas, Kovacs et al. 2008). In northern Greenland and the Canadian Arctic ringed seals primarily feed on codfishes (Boerogadus saida and Arctogadus glacialis), in West Greenland on amphipods (Parathemisto spp. and Themisto spp.), capelin (Mallotus villosus), redfish (Sebastes sp.) and cephalopods, whereas capelin was the main prey in southern Greenland (Siegstad, Neve et al. 1998; Holst, Stirling et al. 2001). Ringed seals may also feed on decapods, euphausiids (e.g. krill), and mysids (Welch, Bergmann et al. 1992; Siegstad, Neve et al. 1998). In the White Sea, the ringed seal diet primarily consists of sand lance (Ammodytes hexapterus), capelin (Mallotus villosus), herring (Clupea harengus), and various crustaceans (Svetocheva 2004). In addition to regional and seasonal variability, foraging strategies and diets tend to vary with age (i.e. size) with older animals exploiting deeper waters, larger prey and/or defending attractive high productivity feeding grounds in fjords and bays (Born, Teilmann et al. 2004; Freitas, Kovacs et al. 2008). Although ringed seals forage year round, foraging is most intense in the open water period during which they recuperate the body mass lost during the breeding and moulting period (Ryg, Smith et al. 1990).

4.1.7 Predation and interspecific competition

Ringed seals form the main diet of and are intensely predated upon by polar bears (Smith and Stirling 1975; Stirling and McEwan 1975; Welch, Bergmann et al. 1992; Derocher, Wiig et al. 2002), and the two species have significantly shaped the behaviour and ecology of each other (e.g. Stirling and McEwan 1975; Ferguson, Taylor et al. 1999; Mauritzen, Derocher et al. 2001; Wiig, Born et al. 2003; Ferguson 2006). Polar bears may remove close to 50% of the annual ringed seal pup production in some areas and years (Hammill and Smith 1989). Arctic fox (Alopex lagopus) typically scavenge on polar bear kills, but may also predate directly on ringed seal pups in birth lairs (Hammill and Smith 1989). Ringed seals and their pups in particular are also predated and/or scavenged by Greenland sharks (Somniosus microcephalus), killer whales (Orcinus orca), and walruses (Odobenus rosmarus), as well as gulls (Larus hyperboreus), ravens (Corvus corax), wolves (Canis lupus), red foxes (Vulpes vulpes), and wolverines (Gulo gulo) in areas where these occur (Lydersen 1998; Reeves 1998; Ridoux, Hall et al. 1998). The flexibility of ringed seal foraging strategies implies that interspecific competition for prey resources is limited, although locally, ringed seals may compete with harp seals (Pagophilus groenlandicus), bowhead whales (Balaena mysticetus), narwhals (Monodon monoceros), and sea birds (Reeves 1998; Born, Teilmann et al. 2004; Cooper, Budge et al. 2009).

4.1.8 Anthropogenic exploitation

Ringed seals have been an important species for subsistence hunting since the peopling of the Arctic and may be the sole reason that Inuit survived during the winter months when other seal species
migrated south (Reeves, Wenzel et al. 1998; Teilmann and Kapel 1998). The harvested ringed seals are used for consumption, fuel, clothing, tool parts and handicraft, and is of continuous cultural and local economic importance to the Canadian and Greenlandic Inuit (Furgal, Innes et al. 2002). The current catch amounts to 50,000 – 80,000 seals per year in Canada (Reeves, Wenzel et al. 1998) and up to 100,000 in Greenland (Teilmann and Kapel 1998). The scattered distribution and general inaccessibility of ringed seals have precluded large scale commercial hunting, as opposed to the harp seals in Canada, and consequently, subsistence hunt on ringed seals is unlikely to pose a threat to the population under the current conditions (Teilmann and Kapel 1998).

4.1.9 Climate change

The extent of sea ice in the Arctic has been decreasing steadily as a consequence of the current global warming (IPCC 2007; Perovich and Richter-Menge 2009), and is have been hypothesized to have large effects upon the distribution and abundance of Arctic marine mammals as well as the species to which they are ecologically linked (e.g. Tynan and DeMaster 1997; Walther, Post et al. 2002; Parmesan 2006; O’Corry-Crowe 2008; Perovich and Richter-Menge 2009; Post, Forchhammer et al. 2009). Ringed seals appear to have evolved an “ecological flexibility” characterized by a high mobility, a broad range of prey species, and an ability to over-winter in ice-covered areas as well as in the pack-ice, which likely confer increased population viability relative to other pinnipeds faced with similar climatic and environmental fluctuations (Born, Teilmann et al. 2004; Laidre, Stirling et al. 2008). Thus, while it seems unlikely that the Arctic ringed seal become extinct or endangered, abrupt habitat changes caused by recent global warming may lead to considerable shifts in its distribution and abundance with subsequent consequences for e.g. polar bears and the Inuit, as well as other species of the Arctic ecosystem to which the ringed seal is ecologically linked (Stirling and McEwan 1975; Welch, Bergmann et al. 1992).

4.2 Objectives

One manner in which to gain insights into the potential ecosystem effects of global warming in the Arctic is to assess the effects of annual sea-ice dynamics on the genetic diversity in Arctic marine fauna. The pagophilic lifestyle of ringed seals indicates that the microevolutionary history of ringed seals might be correlated with the annual sea-ice dynamics of the Arctic. However, given that current populations of Arctic ringed seals likely were established relatively recently (i.e. during the Holocene) relatively precise estimates of effective population sizes, divergence times as well as gene flow among subpopulation likely require more data than has typically been applied in micro-evolutionary studies of natural populations (Davis, Stirling et al. 2008). Accordingly, we set out to identify and characterize
a large number of SNPs in ringed seals. Ideally, estimates of migration rates, effective population size and migration rates derived from SNPs are expected to be less imprecise than those derived from e.g. microsatellite data. However, the identification of many SNPs in non-model species, such as the ringed seal, may be difficult.

The purpose of Part I of this thesis was to develop a novel set of single nucleotide polymorphisms (SNPs) in the ringed seal and subsequently apply evolutionary and population genetic analyses to shed light on the relationship between the annual sea-ice dynamics and the micro-evolution of ringed seals. Understanding the effect of sea-ice upon local abundance and connectivity among regions is important for the assessment of the potential impact of the current climate change on pagophilic marine mammals.

4.3 Results and future directions

Paper I describes a simple method to identify SNPs in non-model species and shows that a relatively large number of SNPs could readily be isolated from the ringed seal without the need for next-generation sequencing facilities, making the methodology available to standard population genetic laboratories.

In addition to the 96 ringed seals characterized in Paper I, DNA has been extracted from more than 1400 ringed seals from Greenland and Svalbard. From this initial set, an additional 1056 ringed seals, providing an adequate spatial and temporal coverage of the study area, were selected for sequencing and SNP genotyping. The resulting data set consists of 400 bp of mtDNA and approximately 2000 SNPs distributed across 96 nuclear loci, each 200-300 bp long, which has yet to been analyzed. As some of the genotype data received from the sequencing provider (Polymorphic Inc.) contained inconsistencies, the raw chromatogram sequence data was set up for a new round of base calling and quality assessment using the Phred-Phrap-Polyphred-Consed pipeline (Nickerson, Tobe et al. 1997; Ewing and Green 1998; Ewing, Hillier et al. 1998; Gordon, Abajian et al. 1998), but I have not yet called the data and hence none of the originally planned micro-evolutionary analyses have been carried out to this date.
5. Molecular background of allozyme variation in fin whales

5.1 The North Atlantic fin whale

5.1.1 Taxonomy and phylogeny

The fin whale (Balaenoptera physalus) is the second largest of the baleen whales only superseded by the blue whale (Balaenoptera musculus) (Figure 4). Fin whales branch together with humpback whales (Megaptera novaeangliae) in molecular phylogenies (Baker, Perry et al. 1993; Nikaido, Hamilton et al. 2006; Steeman, Høgsaard et al. 2009), but may hybridize with the blue whale to produce viable and seemingly fertile offspring (Arnason, Spilliaert et al. 1991; Bérubé and Aguilar 1998). Fin whales are cosmopolitan and occur in all oceans, forming at least three distinct groupings inhabiting the North Atlantic, the North Pacific, and the southern Hemisphere (Donovan 1991). The fin whales of the northern and southern hemisphere are typically recognized as different subspecies B. p. physalus and B. p. quoyi, respectively (SMM 2011), and an additional southern hemisphere subspecies, the pygmy fin whale (B. p. patachonica), has been proposed (Clarke 2004). The following characterization focuses on the North Atlantic fin whale population.

5.1.2 Species description

Fin whales have a long and streamlined body with a small dorsal fin. They are gray/dark on the dorsal side and white on the ventral side. In the North Atlantic, fin whales average at 20 meters in length, but may reach up to 27 meters, and weight between 45 and 75 tonnes, depending on season and individual body condition (Lockyer 1986). Fin whales are more gregarious in behaviour than other baleen whales and are often found travelling in pairs or small groups (Whitehead and Carlson 1988), which may merge and form larger loosely associated male-biased aggregations at the feeding grounds (Bérubé, Berchok et al. 2001).

5.1.3 Distribution, habitat and movements

The North Atlantic fin whale has an extensive distribution, observed at the edges of the Arctic pack ice, southward to the Gulf of Mexico, Azores, Canary Islands, Mediterranean Sea and West Africa,
although they are generally more common north of the 30°N latitude (Rørvik and Jonsgård 1981; Vikingsson, Pike et al. 2009). Fin whales are pelagic and tend to be most abundant over the continental slope and on the shelf seaward of the 200 m isobath (Rørvik and Jonsgård 1981). They feed at high- mid-latitudes and different feeding populations may exhibit differing degrees of movement. Breeding grounds have never been identified, and some animals may overwinter and breed at the mid- to high-latitudes (Rørvik and Jonsgård 1981; Simon, Stafford et al. 2010). The annual migration pattern of fin whales is unknown (Rørvik and Jonsgård 1981; Donovan 1991).

Sighting records of individual animals identified by photo-ID of natural markings (e.g. scars and pigmentation patterns on the front side of the head) (Clapham and Seipt 1991; Whooley, Berrow et al. 2011), mark-recapture studies (Gunnlaugsson and Sigurjónsson 1989) and acoustic data (Delarue, Todd et al. 2009), suggest that certain animals have site fidelity to certain feeding areas, but besides those relating to the predictability of prey abundance, little is known about the environmental or behavioural factors that may drive such fidelity.

The degree of gene flow within and among feeding and possible breeding grounds is uncertain. One genetic study indicates the existence of a genetically distinct Mediterranean population, as well as slight population structuring between eastern and western North Atlantic feeding grounds, resembling a scenario of isolation by distance with male-biased gene flow (Bérubé, Aguilar et al. 1998). The low levels of population genetic structuring may result from some degree of mixing at feeding grounds, or may result from population expansions in the northern North Atlantic following the last glaciation (Bérubé, Aguilar et al. 1998). However, other studies suggest high levels of genetic differentiation among feeding sites, as discussed in Section 5.2.

5.1.4 Life-history

Male and female fin whales attain sexual maturity at the age of 5 to 15 years, but may not be physically mature before the 20 to 30 years of age, possibly depending on food availability and hunting pressure (Aguilar and Lockyer 1987). Males generally grow faster than females, but females are larger than males at physical maturity (Aguilar and Lockyer 1987). The reproductive life-span is unknown, but fin whales may live for 80 years or more (Aguilar and Lockyer 1987). Mature females follow a two year reproductive cycle and bear a single calf every 2-3 years. Mating occurs during the winter and birth of a single c. 6 meter long calf about 11 months later. Weaning takes place at the end of the summer feeding season when the calf is 6-7 months old and measures approximately 12 meters (Aguilar and Lockyer 1987). The mating system of fin whales is unknown.
5.1.5 Foraging, competitors and predators

Fin whales feed during summer in high productivity areas at mid- to high-latitudes. Like other baleen whales, they feed by gulping and filtering large swarms of prey (Lockyer 2007), which allows for the build-up of energy reserves to sustain them through the winter season, during which feeding is assumed to be limited (Vikingsson 1990; Vikingsson 1997). The fin whale diet consist of krill (Euphausiacea) and schooling fish such as capelin (Mallotus villosus), herring (Clupea harengus), and sand lance (Ammodytes spp.) (Skern-Mauritzen, Johannesen et al. 2011). Different baleen whale species often target the same food resources, but although interspecific competition may occur, it appears that their primary prey differ in size, and spatial and/or temporal distribution (Whitehead and Carlson 1988; Ingram, Walshe et al. 2007; Santora, Reiss et al. 2010; Skern-Mauritzen, Johannesen et al. 2011). Killer whales may occasionally take fin whale calves and even adults, but pathogens, pollution, ice-entrapments, entanglement in fishing gear, and ship collisions are probably greater causes of mortality (Aguilar and Lockyer 1987; Hobbs, Muir et al. 2001; Notarbartolo-di-Sciara, Zanardelli et al. 2003).
5.1.6 Anthropogenic impacts

The high swimming speed of fin whales and their tendency to sink when killed meant that they were only targeted occasionally before the onset of modern whaling in the 1860s (Tønnesen and Johnsen 1982). The modern whaling period, however, saw severe declines in fin whale populations with more than 80,000 animals harvested up till the 1980s in the North Atlantic alone (Víkingsson, Pike et al. 2009). High efficiency whaling caused the depletion of the fin whale population off Norway already by 1904, but the Norwegians and others continued to harvest fin whales in other regions of the North Atlantic, where the scenario was repeated; following the establishment of new whaling stations, local fin whale populations were typically depleted within 10-20 years (Tønnesen and Johnsen 1982). For example, whaling off Gibraltar started in 1921 and the population crashed just five years later after the harvest of more than 4000 fin whales (Clapham, Aguilar et al. 2008). By the 1930s, whaling for fin whales in the North Atlantic had become unprofitable and largely abandoned, but was resumed in many areas following the Second World War (Tønnesen and Johnsen 1982). Norway, the Faroe Islands, and Iceland continued commercial harvesting of fin whales up until the IWC moratorium of 1986, after which Iceland operated a scientific whaling program until 1989, taking approximately 300 fin whales (Tønnesen and Johnsen 1982; IWC 2012). Iceland is currently member of the IWC and committed itself not to authorize whaling before 2006, and thereafter not as long as progress is being made in negotiations regarding IWC’s management framework for sustainable whaling; the Revised Management Scheme (RMS) (IWC 2007; IMFA 2009; IWC 2009). Greenland continues to hunt in the order of 10-20 fin whales annually under aboriginal subsistence quotas (NAMMCO 2005). Iceland resumed their whaling program in 2006 under objection to the IWC moratorium and with reference to the lack of progress on the RMS (IMFA 2009). In 2006, the Icelandic harvest was 7 fin whales, and no fin whales have been taking since then (as of the 2008/2009 season) (IWC 2012).

5.1.7 Conservation and management

The North Atlantic fin whale falls under the management jurisdiction of the IWC which together with the North Atlantic Marine Mammal Commission (NAMMCO) assess the management status of fin whales in the North Atlantic (IWC 2007; IWC 2009). The total North Atlantic population is estimated to number 60-70,000 animals, and although it appears to be increasing, at least locally (Víkingsson, Pike et al. 2009), its management status is still listed as uncertain by the IWC (IWC 2007; IWC 2009). A large part of this uncertainty results from an unclear understanding of fin whale population structure and seasonal movements; knowledge which is necessary to delineate demographic independent units (Waples and Gaggiotti 2006; Palsbøll, Bérubé et al. 2007), assess their degree of recovery from
whaling (by estimating their abundance), as well as evaluate the potential effects of resuming harvest of certain feeding populations (as currently proposed by Iceland).

The IWC currently operates with seven distinct North Atlantic fin whale management units based on the geographical separation of feeding areas (i.e. eastern Canada, western Greenland, eastern Greenland, western Iceland, eastern Iceland and the Faroe Islands, Norway, and western Spain) (Donovan 1991; IWC 2007; IWC 2009). However, given their seemingly continuous distribution and unknown winter breeding grounds, as well as highly contradictory results from genetic analyses and an incomplete knowledge of putative environmental and behavioural dispersal barriers, it is widely acknowledged that these delineations are at best preliminary (IWC 2007; IWC 2009).

5.2 Objectives

Much of the uncertainty regarding North Atlantic fin whale population structuring is due to seemingly contradicting results from population genetic analyses (IWC 2007; IWC 2009). Allozyme loci has revealed very high levels of genetic differentiation among summer feeding sites indicative of low migration (Daníelsdóttir, Duke et al. 1991; Daníelsdóttir, Sigurjónsson et al. 1992), whereas presumed neutral genetic markers (i.e. microsatellites and mtDNA) detected comparatively low levels of genetic differentiation, approaching panmixia (Bérubé, Aguilar et al. 1998). The two most likely explanations for the high levels of genetic differentiation at the analyzed allozyme loci are; i) the allozyme loci are under divergent natural selection; or ii) technical issues relating to e.g. differential treatment of samples during collection and storage.

The purpose of Part II of this was to determine the more likely of the two explanations regarding the high divergence in allozyme loci, and hence aid in resolving the uncertainty regarding fin whale population structuring. Our approach was to sequence the exons encoding the most divergent allozyme loci, determine the presence of non-synonymous substitutions, and assess how these potentially relate to the observed allozyme variation. The presence of non-synonymous substitutions is indicative of selection, whereas the absence is indicative of technical issues; or so we thought.

5.3 Results and future directions

Paper II investigates the molecular basis of presumed genetic divergence in allozyme loci among North Atlantic fin whale feeding populations. In sequencing the exons encoding the messenger RNA for the two most divergent allozyme loci (MDH-1 and MPI) we failed to identify non-synonymous SNPs corresponding with the observed allelic variation in the allozyme assay. Following extensive
error checking and analysis of additional bioinformatic and morphological data we hypothesize that the observed allozyme polymorphisms may reflect phenotypic plasticity at the cellular level, perhaps a response to fluctuations in prey density. These findings may provide valuable input to the current debate about the molecular mechanisms that govern local adaptation. Our findings also highlight that allozyme data should be interpreted with caution since the observed genetic divergence may not represent the results of genetic drift and migration, and therefore not reflect population structure.

The hypothesis that emerged from Paper II, that the observed allozyme polymorphisms in fin whale liver cells may result from phenotypic plasticity, will need to be tested. One way to approach the question is RNA-seq (Wang, Gerstein et al. 2009) and mass spectrometry (Hu, Noll et al. 2005). Although associated with logistic (and ethical) difficulties, the fresh liver cells needed for such an analysis could be obtained from harvested fin whales. Regardless of the underlying cause of the observed pattern, it is clear that inference of population structuring in North Atlantic fin whales will require much larger sample sets and number of genetic markers, as well as the adoption of e.g. kinship based approaches to test if the low levels of population divergence observed at mtDNA and microsatellite loci is due to high rates of gene flow or recent divergence (Okland, Haaland et al. 2010; Palsbøll, Zachariah Peery et al. 2010).
6. Telomeres, age and life-history trade-offs in the humpback whale

6.1 The North Atlantic humpback whale

6.1.1 Taxonomy, phylogeny and species description

The humpback whale (*Megaptera novaeangliae*) is a mid-sized baleen whale, belonging to the rorquals and closely related to the fin whale (Figure 5) (Baker, Perry et al. 1993; Steeman, Hebsgaard et al. 2009). There are no recognized subspecies of humpback whale despite its global distribution (SMM 2011). The humpback whale is easily distinguished from other rorquals (and cetaceans in general) by its long pectoral fins, whose length is approximately one third of the entire body (Borowski 1781; Clapham and Mead 1999). The pectoral fin, as well as the area around the mouth, has a number of large tubercles, not seen in other cetaceans. Humpback whales have a dorsal fin approximately two-thirds towards the back of the body. The humpback whale is mainly black with a white-grey ventral side, but individual coloration is highly variable, to the extent that photographs of natural markings on the fluke can be used for individual identification (Katona and Whitehead 1981). Average body length at birth is 4.5 meters, and physically mature individuals measures 13-14 meters, with females being slightly larger than males (Chittleborough 1965).

6.1.2 Distribution, habitat and movements

The humpback whale occurs in all the worlds’ oceans, but is rarely seen in the Mediterranean (Clapham and Mead 1999). It is highly migratory, travelling up to 18,000 km between their seasonal distributions at mid-high latitude feeding areas in the spring, summer and fall, and low latitude breeding areas in the winter (e.g. Palsbøll, Allen et al. 1997; Robbins, Dalla Rosa et al. 2011). In comparison to e.g. the pelagic fin whale, the humpback whale is relatively coastal, typically feeding in near shore waters and/or on shallow offshore banks (Skern-Mauritzen, Johansen et al. 2011), and breeding at Tropical reef systems and islands (Whitehead and Moore 1982; Clapham 1996).

Although inter-oceanic movements have been suggested on both evolutionary (Baker, Palumbi et al. 1990; Palsbøll, Clapham et al. 1995) and ecological time-scales (Palsbøll, Clapham et al. 1995; Pomilla and Rosenbaum 2005), humpback whales are generally considered to be divided into distinct
oceanic populations, particularly in the northern Hemisphere, and some population structuring may occur within oceans (Baker, Palumbi et al. 1990; Baker, Perry et al. 1993; Palsbøll, Clapham et al. 1995; Palsbøll, Allen et al. 1997; Smith, Allen et al. 1999; Rosenbaum, Pomilla et al. 2009). Humpback whales sampled at the feeding grounds segregate in mtDNA into eastern and western stocks within the North Atlantic, North Pacific, South Pacific, South Atlantic, and Indian ocean, consistent with matrilineal directed site fidelity to feeding areas (Baker, Palumbi et al. 1990; Palsbøll, Clapham et al. 1995; Larsen, Sigurjonsson et al. 1996; Valsecchi, Palsbøll et al. 1997; Baker, Medrano-Gonzalez et al. 1998). The population in the Arabian Sea is non-migratory and hence breed and feed in tropical waters (Rosenbaum, Pomilla et al. 2009).

In the North Atlantic, feeding occurs in high-productivity areas in the Barents Sea, off Iceland, west Greenland, and along the eastern US and Canadian sea border (Clapham 1996; Palsbøll, Allen et al. 1997; Smith, Allen et al. 1999). Humpback whales are also observed at the Azores, but have not been studied in great detail in this area (AWWB 2012). The principal North Atlantic breeding site is located in the West Indies (Whitehead and Moore 1982; Palsbøll, Allen et al. 1997), but breeding may also take place to some (unknown) extent at the Cape Verde Islands (Reiner, Santos et al. 1996).

6.1.3 Foraging, competitors and predators

Presumably, the humpback whale feeds exclusively during the summer, and fasten during the annual migrations and throughout the breeding season, during which they rely on stored fat reserves. Humpback whales employ a diverse array of feeding strategies, including bubble net feeding, and prey on krill (euphasiids) and several species of fish, including sand lance (Ammodytes spp.), herring (Clupea spp), mackerel (Scomber scombrus), sardines (Sardinella), and capelin (Mallotus villosus) (Clapham and Mead 1999). In the Barents Sea, and elsewhere, humpback whales share common feeding grounds with fin whales and minke whales, but it is uncertain to what extent they compete for prey resources (Skern-Mauritzen, Johannesen et al. 2011). Killer whales (Orcinus orca) may attack humpback whales and inflict some mortality, in particular on calves, but there is nothing to suggest that predation is a major threat to humpback whales (Clapham 1996; Clapham and Mead 1999).

6.1.4 Anthropogenic impacts, management and conservation

Humpback whales have been hunted in the North Atlantic at least since the 17th century, and these were also among the first to be targeted at the onset of the modern whaling period in the 1860s; presumably because humpback whales are coastal and have relatively low swimming speed compared to other rorquals, and thus are easier to catch (Tønnesen and Johnsen 1982; Clapham and Mead 1999).
By the 1910s, humpback whale stocks in the eastern North Atlantic had become commercially extinct, and the western North Atlantic the stocks were depleted by the 1920s (Tønnesen and Johnsen 1982).

Figure 5 The humpback whale is globally distributed, but occurs at highest abundances at specific feeding grounds in the summer (light blue) and breeding grounds in the winter (dark blue). The population in the Arab Sea appears to be non-migratory (black circle). Top: humpback whales feeding in Gulf of Maine. Notice the baleen. Bottom: Male humpback whales fighting at the breeding grounds in the West Indies. Upper photos: Morten Tange Olsen. Lower photo: Jooke Robbins, with permission. Map: modified from www.wikipedia.org
Hunting in the North Atlantic was banned in 1955 and the humpback whale granted worldwide protection in 1966 (IWC 2012). Since 1986, exploitation has been minimal, with only two animals taken annually at St. Vincent & The Grenadines and in West Greenland under aboriginal subsistence permit (IWC 2012). Since its protection, the North Atlantic population has been growing at a rate of 3-5%, and the most recent population size estimates from 1992-1993 are in the order of 10,000 animals (Smith, Allen et al. 1999). Current threats to humpback whales, and most other large cetaceans, include ship strikes, entanglement in marine debris, and possibly pollution, however the mortality rates are difficult to assess (Clapham and Mead 1999; Clapham, Young et al. 1999; Johnson, Salvador et al. 2005). In 2008, the IUCN changed the global status of the humpback whale from “Vulnerable” to “Least Concern” (IUCN 2012), although some subpopulations (e.g. South Georgia, New Zealand, south-eastern Caribbean) have not recovered from whaling yet (Clapham, Young et al. 1999).

6.1.5 Life-history and mating system

Predictable movement pattern, nearshore habitat, and relatively well-defined feeding and breeding regions have allowed for detailed observations of humpback whale life-history. Humpback whales become reproductively mature around the age of 5 years, but do not attain full physical maturity before they are 10-15 years old (Chittleborough 1965; Clapham and Mayo 1987). The maximum lifespan is unknown, but they may live up to 48 years of age (Chittleborough 1965), or perhaps twice as long (Gabriele, Lockyer et al. 2010), depending on how ear-plug growth layer groups are interpreted. Females come into oestrus during the winter breeding season and gestate for 11-12 months, before giving birth to a single calf (Chittleborough 1965), which is nursed for 6-12 months (Clapham and Mayo 1987).

Mating include mate choice in that both males and females preferentially associate with large individuals of the opposite sex (Pack, Herman et al. 2009). Large females have the advantage that they can accumulate relatively more body fat and thus store greater energy reserves for gestation and lactation and have more efficient thermal regulation and hence effective energy conservation (Clapham 1996). Also, they are presumably better to defend calves against predators and generally older and hence more experienced (Clapham 1996). These advantages presumably entail strong sexual selection for female body size resulting in females being slightly larger than males (Ralls 1976; Ralls and Mesnick 2002).

Unlike many other polygynous mammals, female humpback whales are loosely distributed at the breeding grounds, in a manner described as “floating lek” and consequently a single male cannot monopolize a large group of females, but have to compete with other males for access to a female
Humpback whale telomeres

(Baker and Herman 1984; Clapham 1992; Craig and Herman 1997). Such competitive groups are not observed in other baleen whales, and it is unclear how they form. It has been proposed that males organize themselves hierarchically by their song or to gain mutual assistance in mating (Darling and Bérubé 2001; Darling, Jones et al. 2006), however the function of the male humpback whale song is highly debated and may alternatively function as a sexual display to attract females and/or to warn other males (Payne and McVay 1971; Winn and Winn 1978; Noad, Cato et al. 2000). Competitive groups typically consist of a single female (with or without a calf), a large male termed the principal escort, and a varying number of male secondary escorts and challengers (Clapham, Palsbøll et al. 1992; Spitz, Herman et al. 2002). The active participation in competitive groups is presumably energetically costly to males. However these costs may be outweighed by the benefits of mating and hence males attempt reproduction each season (Chittleborough 1965; Spitz, Herman et al. 2002). In contrast, females bear the maternal costs associated with gestation and production of a very fat-rich milk (Lockyer 2007) and typically follow a 1-3 year mating cycle (Chittleborough 1965), varying slightly with age, body condition, and geographical region (Clapham and Mead 1999). Hence, reproduction may be costly to both males and females and entail trade-offs between reproduction, growth, and self-maintenance, however the life history strategies for dealing with these costs are unknown and perhaps subject to inter-individual and lifetime variations.

6.2 Objectives

Given the need and wide-ranging applications of a non-lethal method for age determination of free-ranging whales, as well as the wide availability of samples from known-age individuals, this part of my thesis aimed at developing a qPCR based telomere assay for humpback whales and subsequently evaluate whether the correlation between telomere length and age is strong enough to be used for age determination. Moreover, progress in the study of human telomeres indicates that telomeres may be good proxies of individual life histories, fitness and expected lifespan. Hence, a second aim of this part of my thesis was to apply telomeres for estimating correlations between life history parameters and the costs of reproduction in humpback whales.

6.3 Results and future directions

Paper III present and evaluate the performance of four different telomere qPCR assays for measuring relative telomere lengths in humpback whales. We found that even assays performing well by existing qPCR standards failed when subject to more extensive quality control, suggesting that commonly used quality criteria may be inadequate and that more stringent criteria should be applied to telomere research and qPCR applications in general.
**Paper IV** applies the most precise and accurate of the four qPCR assays evaluated in **Paper III** to assess the potential for genetic age determination of humpback whales. We found that telomeres lack the necessary resolution even for approximate age determination. This was mainly due inter-individual variation making it impossible to derive a simple and sufficiently precise relationship between telomere length and age. We conclude that although telomeres cannot be used for age determination they may provide interesting insights into individual and species specific life-history characteristics.

Finally, **Paper V** addresses fundamental life history questions regarding the costs of reproduction in long-lived mammals by integrating individual rates of telomere shortening in humpback whales with gender and reproductive output. As would be predicted from life-history theory, we found that the costs of reproduction, quantified in terms of the rate of telomere shortening, are higher in mature humpback whales than in juveniles; that reproductive costs are higher in males than females; and that differences among females in the rate of telomere loss tended to correlate with reproductive output during the sampling period.

The results of the telomere project indicate that telomeres may be powerful markers of individual and species life-history trade-offs with wide-ranging applications within physiology, ecology and evolutionary biology. Broad scale applications of telomeres in the study of non-model species would benefit from a less sensitive approach to telomere length estimation than the multiplex qPCR method (Cawthon 2009). A first step towards this goal could be to compare the performance of the qPCR method with that of the recently described dot blot method (Kimura and Aviv 2011) in a manner similar to the recent comparison of the qPCR and TRF method performed by Aviv and co-authors (2011). Second, future applications would also benefit from a comparison of relative telomere lengths measured in different tissues of the same individual to establish whether telomere length are similar in biopsied skin tissue to that of other tissue and cell types. One way to test this in cetaceans would be to obtain samples from the humpback whale and fin whale (*Balaenoptera physalus*) catch in Greenland and Iceland, respectively. Third, from a marine mammal perspective it would be interesting to compare telomere dynamics of different baleen whale species characterized by different life histories and habitats, e.g. bowhead whale (*Balaena mysticetus*), minke whale (*Balaenoptera acutorostrata*), Bryde’s whale (*Balaenoptera breydei*), and fin whale (*Balaenoptera physalus*) or blue whale (*Balaenoptera musculus*) in order to test predictions regarding cost of reproduction and migration (e.g. the highly migratory humpback whale versus a non-migrating Bryde’s whale), longevity (e.g. long-lived bowhead whale versus the presumably short-lived minke whale), and body size (the small minke whale versus the large fin or blue whale). Finally, given that cancer cells have unlimited proliferative ability due to high telomerase activity (Blackburn 1991) and that cetaceans are long-lived but characterized by an apparent absence of cancer (Caulin and Maley 2011) further insights in cancer
biological studies may be obtained by investigating the underlying physiological processes governing telomere maintenance in cetaceans, e.g. by using cultured cells from free-ranging or harvested animals.
7. Contributions

Paper I

I performed the sub-sampling of ringed seals from freezers in Denmark, extracted all DNA, was responsible for; (i) communication with the sequencing centre Polymorphic Inc., (ii) data quality control and trouble-shooting, (iii) data analysis, and writing of the manuscript as well as annual and final reports to the National Science Foundation. Moreover, I did install and test the Unix programs necessary to conduct the base-calling with assignment of quality scores as implemented in the genotyping pipeline. The conceptual framework (hypothesis, species and markers) was conceived by my supervisors who also obtained the funding from NSF. The initial cloning, sequencing and identification of the SNPs (prior to re-sequencing) described in the manuscript was conducted by VHV when a post-doctoral researcher with PJP at UC Berkeley.

Paper II

The project (objective, species and approach) was conceived by my supervisors, whereas I designed the experimental set-up, performed the vast majority of the experimental work associated with sequencing the MDH-1 and MPI genes (i.e. primer design and optimization, PCRs and cycle sequencing), genotyped and analyzed all DNA sequence and protein data, performed all bioinformatic analyses, and wrote the manuscript. I did not perform the experimental work and genotyping associated with the allozyme and microsatellite markers, which was done by AKD, MB and CP, but I did carry out all subsequent analyses of the allozyme and microsatellite genotype data.

Papers III, IV and V

The funding for the telomere age determination was granted by the US Marine Mammal Commission to my supervisors in response to a proposal to investigate if telomeres could be used for age determination in baleen whales written by Mary Beth Rew, at the time a graduate student of my supervisors when at UC Berkeley. I designed and conducted all the experimental work described in the three manuscripts. The idea to describe and evaluate the different telomere qPCR assays in a methodological paper was conceived by me, as well as the hypothesis that the rate of telomere length reduction might correlate with other life history traits. I wrote all three manuscripts as well as the preliminary and final reports to the US Marine Mammal Commission.
8. Epilogue

The diversity and distribution of extant marine mammals primarily result from periodic bursts of geological and climatic change to which these and other organisms have responded by shifting their geographical distribution, timing or life history events (e.g. growth and reproduction), phenotypic plasticity, adapting, or becoming extinct. These are processes of natural selection. Given the increase in anthropogenic impacts during the 19th and 20th century and the resulting severe population declines in most marine mammal populations, there is a concern what the future might bring for marine mammals as habitat fragmentation, pollution and other anthropogenic impacts put additional pressure on local and global populations (Kaschner, Tittensor et al. 2011). Molecular ecology holds great promise for understanding the evolutionary, ecological and demographical effects of environmental changes, and may also aid in shedding light on the effects of anthropogenic impacts on marine mammal abundance and distribution. Genomics have just entered the stage, and soon, exome capturing (Bashiardes, Veile et al. 2005), RNA-seq (Wang, Gerstein et al. 2009), high-resolution mass spectrometry (Hu, Noll et al. 2005), and other novel molecular and analytical techniques are likely to increase our understanding of genetic, physiological, and metabolic responses to environmental change. Although simpler in approach, the work presented here further illustrates the wide utility of molecular ecology in the study of marine mammals.
9. References


References


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References


10. Appendix

Additional publications and manuscripts (2007-2012)


V. Palsbøll PJ, Peery MZ, **Olsen MT**, Bérubé M (manuscript) Inferring historic abundance from current genetic diversity: understanding the nature of the beast. *Molecular Ecology (invited review)*

Conference and workshop presentations (2007-2011)


**Olsen MT** Molecular tools for the study of Cetaceans (and Pinnipeds). Student workshop on tools in marine mammal science. The 25th Annual Conference of the European Cetacean Society, March 2011, Cádiz, Spain. **INVITED TALK.**