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GENETIC POPULATION STRUCTURE OF GRAY WHALES (*ESCHRICHTIUS ROBUSTUS*)

THAT SUMMER IN CLAYOQUOT SOUND, BRITISH COLUMBIA

by

Tammy Elaine Steeves

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in

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# UMI

# GENETIC POPULATION STRUCTURE OF GRAY WHALES (*ESCHRICHTIUS ROBUSTUS*) THAT SUMMER IN CLAYOQUOT SOUND, BRITISH COLUMBIA

BY

Tammy Elaine Steeves

## ABSTRACT

To determine the sex composition of gray whales that summer in Clayoquot Sound (CS), British Columbia and to assess whether these animals represent a genetically distinct subgroup of the eastern North Pacific (ENP) population, I collected skin samples from 18 individuals in CS ("residents"). Fourteen samples obtained from other areas served as random representatives of the overall population in the ENP ("non-residents"). Sex was determined for each sample; the nucleotide sequence of a 311 base-pair segment at the 5' end of the mtDNA control region was determined by automated sequencing. The sex ratio among residents was 2.6 to 1 nominally biased towards males, but was not significantly different from parity ($p=0.06$). Residents were not significantly different from non-residents ($Kst=-0.02$, $p=0.79$). Neighbor-joining analysis revealed three clades that did not correspond to any obvious geographic pattern. These data indicate the ENP population is genetically homogeneous.

## ACKNOWLEDGMENTS

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I also would like to thank Kelly Roberston at the Southwest Fisheries Science Center (SWFSC) for her patience and expertise regarding permits. A special thanks to SWFSC, the Natural History Museum of Los Angeles County and Sea World Parks for providing archived samples.

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# CHAPTER 1

## BACKGROUND AND NATURAL HISTORY

Gray whales (*Eschrichtius robustus*) occur only in the North Pacific although animals once existed on either side of the North Atlantic (Rice and Wolman 1971, Jones et al. 1984). Gray whales were present in the western North Atlantic until the 18th century whereas animals in the eastern North Atlantic are known only from subfossil remains (Mead and Mitchell 1984). The historic population structure of North Atlantic gray whales is unknown. The North Pacific population has generally been divided into two stocks for management purposes. The California-Chukchi stock, also known as the eastern North Pacific population, migrates between calving and breeding grounds on the west coast of the Baja California peninsula to feeding areas in the northern Bering and Chukchi seas (Scammon 1874, Pike 1962, Rice and Wolman 1971, Braham 1984). The Korean-Okhotsk stock, or the western Pacific population, migrates from unknown calving and breeding grounds (possibly along the coast of China) to feeding grounds in the Okhotsk Sea (Scammon 1874, Rice and Wolman 1971, Henderson 1990, Vladimirov 1994). The traditional division of the North Pacific population into these two stocks remains to be tested with genetic or other data.

Both populations were depleted by extensive historical whaling

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operations. The western Pacific population was hunted almost continuously from the late 16th century until at least 1966 (Berzin et al. 1995, Kato andKasuya in press) whereas the eastern North Pacific gray whale population was depleted by two relatively short periods of whaling in the mid 19th and early 20th centuries (Gilmore 1955, Henderson 1984). The western North Pacific population remains small, and its current size is estimated at approximately 250 animals (Vladimirov 1994). In contrast, the eastern North Pacific population, with approximately 21,000 animals, has increased its numbers to near or above historic levels (Henderson 1984, Buckland et al. 1993). The recovery of the eastern North Pacific population prompted its removal from the U.S. List of Endangered and Threatened Wildlife in 1994 (Federal Register 1994). The western North Pacific population remains listed as endangered (Federal Register 1994). The following discussion will be restricted to the eastern North Pacific population.

<u>Biological Parameters</u>

IWC (1993) reviewed estimates of gray whale growth and reproductive parameters. The majority of these estimates were derived from data obtained by examining animals taken near the Chukotka Peninsula since 1965, during the native Chukotka subsistance harvest (e.g. Berzin 1984, Yablokov andBogoslovskaya 1984) and along the coast of central California from 1959 to 1969, during studies conducted under Special Scientific Permits (Rice and Wolman 1971). They can be summarized as follows: the mean birth length for

both sexes is 4.6 meters. Calves reach an average length of 7.0 meters when they are weaned at approximately 5 months and are on average eight meters long at one year. Both sexes reach a median age of sexual maturity at 9 years (range 6 to 12 years). The estimated length at sexual maturity for males and females is 11.1 and 11.7 meters, respectively. Physical maturity is reached by both sexes at approximately 40 years. The average body length at this time for males and females is 13.0 and 14.1 meters, respectively. It should be noted that IWC (1993) recommended reanalysis of the following biological parameters: the length and age at which calves are weaned, body length at one year, and the length and age of sexual maturity.

## Migration

The coastal migration of the gray whale spans 50 degrees of latitude and has often been described as the longest of any mammal (e.g. Rice and Wolman 1971, Jones et al. 1984). The swimming distance between the southernmost breeding lagoon and the northernmost feeding area is approximately 8500 km. However, the longest recorded distance travelled by a gray whale is only 6680 km and was obtained from one animal radio tagged in Laguna San Ignacio, Mexico and later recorded at Unimak Pass, Alaska (Mate and Harvey 1984). The longest recorded distance travelled for any mammal is 8334 km which was obtained from photo-identification pictures of a humpback whale which migrated between feeding grounds off the Antarctic Peninsula and breeding grounds in Columbia (Stone et al. 1990). Palsbøll et al. (1997) used genetic

tagging to record movements of 6435 and 7940 km for two humpback whales

which travelled between breeding grounds in the West Indies and feeding

grounds in the Barents Sea.

In general, gray whales spend January and February on the breeding

and calving grounds, migrate northward from March through May, remain on the

feeding grounds from June through October and migrate southward in

November and December (Pike 1962, Jones et al. 1984). During the northward

migration the majority of the population follows the coastline from Baja

California, Mexico until the animals reach Nunivak Island, Alaska where they

travel offshore to St. Lawrence Island and major feeding grounds in the

northern Bering and Chukchi Seas (Pike 1962, Rice and Wolman 1971,

Braham 1984). The southward migration generally follows the same route,

although the animals may travel farther offshore (Herzing and Mate 1984, Rugh

1984).

Both the spring and fall migration are segregated by age, sex and

reproductive condition (Rice and Wolman 1971). The northward migration

begins with newly pregnant females followed in order by adult males, anestrous

females, immature females, immature males and postpartum females. The

southward migration begins with late-pregnant females followed in order by

recently ovulated females, immature females, adult males and immature males

(Rice and Wolman 1971). The northward migration is further characterized by

two peaks. The first peak (phase A) consists of animals without calves and the

second peak (phase B) consists of cow-calf pairs (Poole 1984, Herzing and

Mate 1984, Braham 1984).

## Breeding and Calving Grounds

There are four main breeding and calving areas on the west coast of the

Baja California peninsula.  They are, from north to south, Laguna Guerrero

Negro, Laguna Ojo de Liebre, Laguna San Ignacio, and the Bahia Magdalena

complex (Rice et al. 1981).  The most comprehensive study was conducted in

Laguna San Ignacio from 1978-1982 (Jones 1984, Jones and Swartz 1984,

Swartz 1986, Jones 1990).  However, the majority of trends observed in the

lagoon also occur in the other areas (Rice et al. 1981, Jones and Swartz 1984).

The abundance and distribution of gray whales in the breeding lagoons

can be divided into two periods.  The first period occurs from late-December to

late-February when both courting whales (males, mature females and immature

animals) and females with calves are present.  During this time, courting

animals are found in the outer lagoons closer to the open ocean whereas cow-

calf pairs are concentrated in the inner lagoon nurseries (Jones and Swartz

1984, Swartz 1986).  The second period occurs from early March until late April

after the single whales have departed and only cow-calf pairs are present.

During this time, females and their calves gradually move down to the outer

lagoons (Jones and Swartz 1984, Swartz 1986).

Courting gray whales begin to arrive in the lagoons in late-December

and reach their maximum abundance in mid-February (Jones and Swartz

1984). Although single whales may remain in the area until mid-March, the majority leave the lagoons and begin their northward migration in mid-February (Jones and Swartz 1984). Photo-identification studies and daily counts indicate that there is a high turnover rate of courting whales within the lagoons and that there is likely a relatively rapid exchange of single animals between breeding lagoons (Jones and Swartz 1984, Swartz 1986).

Most gray whale births occur between late December and early March (Jones and Swartz 1983). The mean birth date is approximately 27 January and the majority of births occur by 15 February (Rice et al. 1981). The majority of cow-calf pairs remain on the breeding grounds until mid-March although some animals may remain in the area as late as early May (Rice et al. 1981, Jones and Swartz 1984, Swartz 1986). Some females and their calves circulate between lagoons prior to their departure to northern feeding areas (Swartz 1986). In particular, Laguna San Ignacio appears to serve as a staging area for numerous cow-calf pairs (Jones and Swartz 1984, Swartz 1986). Maximum cow-calf counts occur in the lagoon in mid-March and field observations demonstrate that the influx is not due to continued births because the late-season calves are approximately 2 to 3 months old (Jones and Swartz 1984). Photo-identification studies further indicate that animals are immigrating to San Ignacio from more southern and more northern breeding lagoons (Jones 1984, Swartz 1986).

The average length of the calving interval is 2 years, although

considerable individual variation exists (Jones 1990). Adult female gray whales alternate between longer stays on the breeding grounds during calving years and shorter stays during non-calving years (Jones and Swartz 1984). Jones (1984) presented three patterns of occupation for breeding females in Laguna San Ignacio: "residents" which spend long periods of time in the lagoon in calving years and short periods of time in non-calving years, "transients" which immigrate to the lagoon with an older calf for a short period of time late in the season and "combination" females that spend long periods of time in the lagoon in some calving years, short periods of time in non-calving years and short periods of time with an older calf late in the season in other calving years. Thus, it is apparent that some females utilize different lagoons in different years and others may switch lagoons within the same year (Jones 1984).

In his review of gray whale migratory, social and breeding behavior, Swartz (1986) stated that the most prominent feature of gray whale behavior is the temporal and spatial segregation of the population into females with calves and whales without calves (males, mature females and immature animals). Females appear to alternate biennially between two behavioral strategies that have important implications for their energy requirements (Jones and Swartz 1984, Swartz 1986). Newly pregnant females leave the breeding grounds earlier and remain on the feeding grounds longer than females that give birth to calves during the same year (Rice and Wolman 1971). As a result, pregnant females spend approximately 7 months on the northern feeding grounds

whereas non-pregnant females only spend about 4 months (Swartz 1986).

Males, however, spend the same amount of time (approximately 5 months)

feeding each year (Jones and Swartz 1984, Swartz 1986). The extra amount of

time spent feeding by pregnant females is of obvious significance given their

need to acquire more energy reserves for their approximate 13.5 month

gestation period (Rice 1983) and subsequent 5 - 7 month lactation period (IWC

1993).

The principal activities of cow-calf pairs on the breeding grounds are

resting, nursing and moving with the changing tides whereas single whales are

predominately engaged in courtship and mating behavior (Swartz 1986).

However, it should be noted that it is not clear to what extent the sexual activity

observed on the 'breeding' grounds actually results in conception. Rice and

Wolman (1971) used the developmental state of embryos and early fetuses

from females collected during the northward migration to estimate a mean

conception date of 5 December (range late November - early January).

Although some females may undergo a second estrous cycle if they fail to

conceive during their first cycle, this estimate suggests that the majority of

females conceive during the southward migration and not on the breeding

grounds as the behavioral data suggests (Rice and Wolman 1971, Rice 1983).

## Northern Feeding Grounds

Several investigators have examined the abundance and distribution of

gray whales on their major feeding grounds in the northern Bering and Chukchi

seas. North American researchers have concentrated their efforts to the northeastern Bering and the eastern Chukchi seas off Alaska (Braham 1984, Moore and Ljungbald 1984, Moore et al. 1986a, Moore et al. 1986b, Clark et al. 1989) whereas Russian researchers have conducted their studies in the northwestern Bering and the southwestern Chukchi seas off the Chukotka Peninsula (Votrogov and Bogoslovkaya 1980, Bogoslovskaya et al. 1981, Bogoslovskaya et al. 1982, Blokhin 1986a, Blokhin 1986b, Blokhin 1989). Studies have been limited to both aerial and ship surveys (Braham 1984), aerial surveys only (Moore and Ljungbald 1984, Moore et al. 1986a, Moore et al. 1986b, Clark et al. 1989), ship surveys only (Votrogov and Bogoslovkaya 1980), shore-based surveys (Blokhin 1986b), or have occurred in conjunction with the native Chukotka subsistence harvest (Bogoslovskaya et al. 1981, Bogoslovskaya et al. 1982, Blokhin 1986a, Blokhin 1989).

The arrival and departure of gray whales to and from the feeding grounds are closely connected to annual movements of the arctic ice (Maher 1960, Votrogov and Bogoslovskaya 1980, Clarke et al. 1989). During the winter, the ice extends south over the Chukchi and northern Bering seas to latitude 60° N (Maher 1960). The ice begins to recede in the spring and by early July open water extends into the southern Chukchi Sea to latitude 70° N (Maher 1960). Substantial numbers of whales enter the northern Bering Sea at the beginning of June (Berzin 1984, Braham 1984) and many continue to follow the receding ice and arrive in the southern Chukchi Sea in early July (Clarke et al. 1989).

Animals remain on the feeding grounds until they move southward from mid-October to November as the ice advances (Votrogov and Bogoslovskaya 1980, Clarke et al. 1989).

Gray whales are found in both shallow inshore waters (< 25 m) and deeper offshore waters (50 - 60 m) in the northern Bering and Chukchi seas (Yablokov and Bogoslovskaya 1984, Moore and Ljungblad 1984). However, spatial segregation of the population does appear to exist to some extent. Investigations of Russian whaling records off the Chukotka Peninsula demonstrate that small animals and females with calves tend to congregate around the shallow waters of the Koryak coastline whereas larger whales are generally found further offshore (Votrogov and Bogoslovskaya 1980, Yablokov and Bogoslovskaya 1984, Blokhin 1989). Most adult males appear to feed in the southwestern Chukchi Sea and more adult females tend to be found in the northwestern Bering Sea (Blokhin 1989). Pregnant females are found at greater depths where prey density is presumably the greatest whereas non-pregnant females appear to be more evenly distributed (Blokhin 1989). Aerial surveys conducted by Moore et al. (1986b) and Clark et al. (1989) off Alaska indicate that more cow-calf pairs are found in the shallow inshore waters of the northeastern Chukchi Sea than in the northeastern Bering Sea.

The most prominent activity in northern waters is feeding (Bogoslovskaya et al. 1981, Moore and Clarke 1986a, Clarke et al. 1989). Additional behaviors include swimming, resting, milling and breaching (Moore and Clarke 1986a,

Clarke et al. 1989). The most stable social units on the northern feeding

grounds are single and paired whales (Bogoslovskaya 1986). Groups of three

to six animals occur but not do not persist for any length of time (Bogoslovskaya

1986). Immature males tend to be found alone whereas mature males occur

more often in groups (Blokhin 1985, 1986a). Conversely, immature females

tend to be found in groups whereas mature females are more often alone

(Blokhin 1985, 1986a). Given these observations, it is apparent that the

segregation of gray whales by age, sex and reproductive condition appears to

be a behavioral characteristic that persists, albeit more loosely on the northern

feeding grounds, throughout the species range (Swartz 1986).

## Southern Feeding Grounds

Although most gray whales in the eastern North Pacific population

migrate to feeding grounds in the Bering and Chukchi seas (Rice and Wolman

1971), some terminate the migration in lower latitudes and spend the summer

along the coasts of California, Oregon, Washington, British Columbia and

Alaska (Gilmore 1960, Pike 1962, Rice and Wolman 1971, Braham 1984,

Darling 1984, Sumich 1984, Mallonée 1991, Weitkamp et al. 1992). Several

studies have examined feeding behavior of the animals within these summer

groups (Murison et al. 1984, Oliver et al. 1984, Guerrero 1989, Mallonée 1991,

Weitkamp et al. 1992). However, the most comprehensive investigation of gray

whale distribution, abundance and behavior has been conducted in Clayoquot

Sound, British Columbia from 1972 to present (Hatler and Darling 1974, Darling

1978, Darling 1984, Darling et al. in press).

Long-term sighting studies in Clayoquot Sound indicate that several whales (approximately 30 individuals) exhibit long-term fidelity to this particular feeding site (Darling 1978, 1984). Adults are typically identified over multiple years whereas young whales are usually present for one to two seasons only (Darling 1984, Darling et al. in press). Within a season, animals may travel into, within and out of Clayoquot Sound which suggests that they are likely part of a larger northwestern coast population (Darling 1978, 1984). Recent photo-identification matches support this idea; several Clayoquot Sound residents have also been identified feeding along the central mainland coast of British Columbia and off the Washington coast (J. Calambokidis, J. Darling and V. Deecke, unpublished data).

Clayoquot Sound is approximately half-way the between southern breeding and northern feeding grounds. The first northbound migrants pass the Sound in mid-February and their numbers peak during last 2 weeks of March although a few whales may still be moving north in May and early June (Darling 1978, 1984). The earliest identified summer residents arrive in late March and early April and remain in the area until the southbound migration begins in late November (Darling 1978, 1984). The peak of the southbound migration occurs during the last two weeks of December but whales can be sighted until late January (Darling 1978, 1984).

Whales within each of the summer groups are generally found within

100-500 m from shore (Sumich 1984, Darling 1978, 1984, Mallonée 1991,

Weitkamp et al. 1992). Feeding is the predominant behavior (Sumich 1984;

Darling 1978, 1984; Mallonée 1991; Weitkamp 1992). Additional behaviors

include travelling, milling and resting (Mallonée 1991; Darling 1978, 1984).

Darling (1978, 1984) also describes "rubbing" behavior that occurs regularly in

a tidal rip-sill area in Clayoquot Sound.

Both young animals and adults occur in Clayoquot Sound (Darling 1978,

1984). Age-separation exists to varying degrees. In its more subtle form, small

(< 8m), presumably very young animals are generally found closer to shore,

often a few hundred metres away from adult assemblages, and appear to have

an affinity for kelp beds (Darling 1978, Darling et al. in press). In its more

obvious form, young animals occupy markedly different habitat over 10 km

inland from feeding adults (Hatler and Darling 1974, Darling et al. in press).

The latter case is exemplified by the sporadic presence of several young

whales in a shallow inland bay (Grice Bay). Hatler and Darling (1974) first

reported one small animal in the area for at least 10 days during the summer of

1971. Two animals were present in Grice Bay during 1984; one of the two

stayed the winter and utilized the area again during the summer of 1985

(Darling et al. in press). In 1995, one to five whales utilized the bay throughout

the summer (Darling et al. in press). One of the five returned for the summer of

1996 (Darling et al. in press, Darling unpublished data). It is not clear how

these young whales "find" Grice Bay and the relationship between them and the

other animals in Clayoquot Sound has yet to be determined (Darling et al. in press). Cow-calf pairs are observed in Clayoquot Sound but they do not tend to remain in the area for prolonged periods of time (Darling 1978, Darling 1984, Darling unpublished data).

The sex of the majority of whales in the Clayoquot Sound summer group is unknown (Darling unpublished data). However, it appears that the group structure is similar to that which is observed on the northern feeding grounds; whales are most often observed alone but they may also be found feeding and travelling in pairs (Darling 1978). Groups of three animals occur, but they are not as common and are less stable (Darling 1978).

Despite over decades of photo-identification research in Clayoquot Sound, the relatedness of the whales within this summer group is unknown. Furthermore, it is not known whether fidelity to Clayoquot Sound persists over many generations, or over a sufficient amount of time to be reflected in the genetic structure of the eastern North Pacific population; such long-term fidelity has been shown for other mysticetes, notably humpback whales (Palsbøll et al. 1995, Larsen et al. 1996).

CHAPTER 2

INTRODUCTION

Gray whales (*Eschrichtius robustus*) occur only in the North Pacific
although animals once existed on either side of the North Atlantic (Rice and
Wolman 1971, Jones et al. 1984). Gray whales were present in the western
North Atlantic until the 18th century whereas animals in the eastern North
Atlantic are known only from subfossil remains (Mead and Mitchell 1984). The
historic population structure of North Atlantic gray whales is unknown. The
North Pacific population has generally been divided into two stocks for
management purposes. The California-Chukchi stock, also known as the
eastern North Pacific population, migrates between calving and breeding
grounds on the west coast of the Baja California peninsula to feeding areas in
the northern Bering and Chukchi seas (Scammon 1874, Pike 1962, Rice and
Wolman 1971, Braham 1984). The Korean-Okhotsk stock, or the western
Pacific population, migrates from unknown calving and breeding grounds
(possibly along the coast of China) to feeding grounds in the Okhotsk Sea
(Scammon 1874, Rice and Wolman 1971, Henderson 1990, Vladimirov 1994).
The traditional division of the North Pacific population into these two stocks
remains to be tested with genetic or other data.

Both populations were depleted by extensive historical whaling

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operations. The western Pacific population was hunted almost continuously

from the late 16th century until at least 1966 (Berzin et al. 1995, Kato and

Kasuya in press) whereas the eastern North Pacific gray whale population was

depleted by two relatively short periods of whaling in the mid 19th and early

20th centuries (Gilmore 1955, Henderson 1984). The western North Pacific

population remains small, and its current size is estimated at approximately 250

animals (Vladimirov 1994). In contrast, the eastern North Pacific population,

with approximately 21,000 animals, has increased its numbers to near or above

historic levels (Henderson 1984, Buckland et al. 1993). The recovery of the

eastern North Pacific population prompted its removal from the U.S. List of

Endangered and Threatened Wildlife in 1994 (Federal Register 1994). The

western North Pacific population remains listed as endangered (Federal

Register 1994).

Although most gray whales in the eastern North Pacific population

migrate to feeding grounds in the Bering and Chukchi seas (Rice and Wolman

1971), some terminate the migration in lower latitudes and spend the summer

along the coasts of California, Oregon, Washington, British Columbia and

Alaska (Gilmore 1960, Pike 1962, Rice and Wolman 1971, Braham 1984,

Darling 1984, Sumich 1984, Mallonée 1991, Weitkamp et al. 1992). Long-term

photo-identification studies in Clayoquot Sound, BC (Figure 1) indicate that

approximately 30 individuals exhibit long-term fidelity to this particular feeding

site (Hatler and Darling 1974; Darling 1978, 1984; Darling et al. in press).
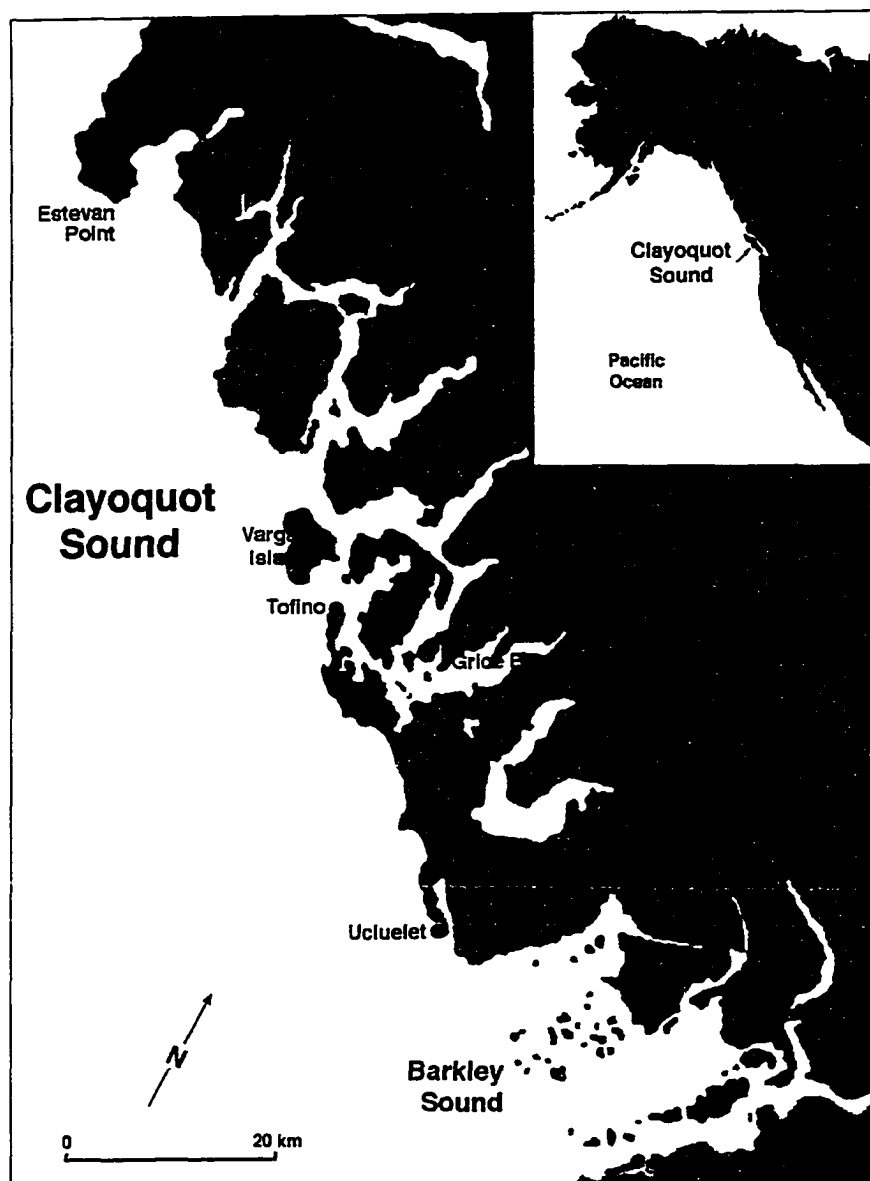
Figure 1. Map of Clayoquot Sound.

Adults are typically identified over multiple years whereas young whales are usually present for one to two seasons only (Darling 1984, Darling et al. in press). The sex composition of these animals is largely unknown. Furthermore, it is not known whether fidelity to Clayoquot Sound persists over many generations, or over a sufficient amount of time to be reflected in the genetic structure of the eastern North Pacific population; such long-term fidelity has been shown for other mysticetes, notably humpback whales (Palsbøll et al. 1995, Larsen et al. 1996).

In this study, I use genetic data to determine the sex composition of gray whales that summer in Clayoquot Sound, and to assess whether these animals represent a genetically distinct subgroup of the eastern North Pacific population.

# CHAPTER 3

## METHODS

### Definitions

Gray whales that summer in Clayoquot Sound have been classified as either "adults" or "young whales" using photogrammetry and other visual estimates of size (e.g. Darling 1978). In this study, animals were classified as adults if sighting data indicated their minimum age was greater than nine years, which is the median age of sexual maturity for both sexes (IWC 1993). An additional classification criterion involved visual estimates of size by comparison to an object of known length (a boat): if an animal was estimated to be approximately 12 meters or greater, it was considered sexually mature (the estimated length at sexual maturity for males and females is 11.1 and 11.7 meters, respectively (IWC 1993, Rice and Wolman 1971)). Animals were defined as "young whales" if visual estimates of their size were less than or equal to eight meters, which is the estimated body length at one year (IWC 1993). I acknowledge that visual estimates of size are relatively poor indicators of maturational status, therefore the classifications of adults based solely on size estimates must be considered tentative.

Approximately 65% of the adult gray whales photo-identified in Clayoquot Sound each year have been observed in previous summers;

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approximately 35% are seen in one year only (Darling 1984,

Darlingunpublished data). The fidelity and duration of stay for the animals that

are sighted in multiple years are variable: some individuals return every year

whereas others may be absent for as many as five years before being re-

sighted; within any season, some individuals may remain in Clayoquot Sound

from the peak of the northbound migration to the peak of the southbound

migration (approximately eight to nine months) whereas others may be

observed sporadically throughout the summer and others still may be present

only for a few weeks at the beginning, middle or end of the season (Darling

1984, Darling unpublished data). Young whales are usually present for one to

two summers only; their duration of stay is also variable (Darling 1984, Darling

et al. in press).

These observations suggest that ther are three different "types" of

summer residents in Clayoquot Sound; adults which are sighted over multiple

years, adults which are sighted in one year only and young whales which are

sighted for one to two years only. Previous studies (e.g. Darling 1984) have

considered all of these animals "summer residents". In this study, a "resident" is

an individual gray whale that has spent at least one summer in Clayoquot

Sound. Residents are subdivided into adults and young whales. Adults are not

further subdivided into adults which are sighted over multiple years and adults

which are sighted in one year only due to the small sample size of this study.

## Sample Collection and Storage

Using the pneumatic rifle biopsy system described by Barrett-Lennard et al. (1996), I collected skin biopsy samples from 18 individual gray whales that summer in Clayoquot Sound (residents). This biopsy system has been used successfully for collecting biopsy samples from killer whales and provoked only mild, short-term reactions in gray whales (T. Steeves and J. Darling, unpublished data). Samples were collected from both adult (n=14) and young (n=4) whales. The sighting history for these animals is summarized in Figure 2. An additional 14 tissue samples were either collected during the northbound migration past Clayoquot Sound (n=1) or obtained from archived material (Southwest Fisheries Science Center, La Jolla, CA, n=6; Natural History Museum of Los Angeles County, Los Angeles, CA, n=5; Sea World Parks, San Diego, CA, n=2). These samples served as random representatives of the overall population in the eastern North Pacific ("non-residents"). Photo-identification records were not available for any of the archived samples. However, the stranding date and location of each of the samples in addition to the fact that there are approximately 21,000 animals in the eastern North Pacific population (Buckland et al. 1993) suggest that it is very unlikely that any of these individuals are residents.

Prior to biopsy sampling in Clayoquot Sound, all animals were individually identified by photographs of natural markings on their sides (Darling 1984). The sighting history for each of the sampled whales was
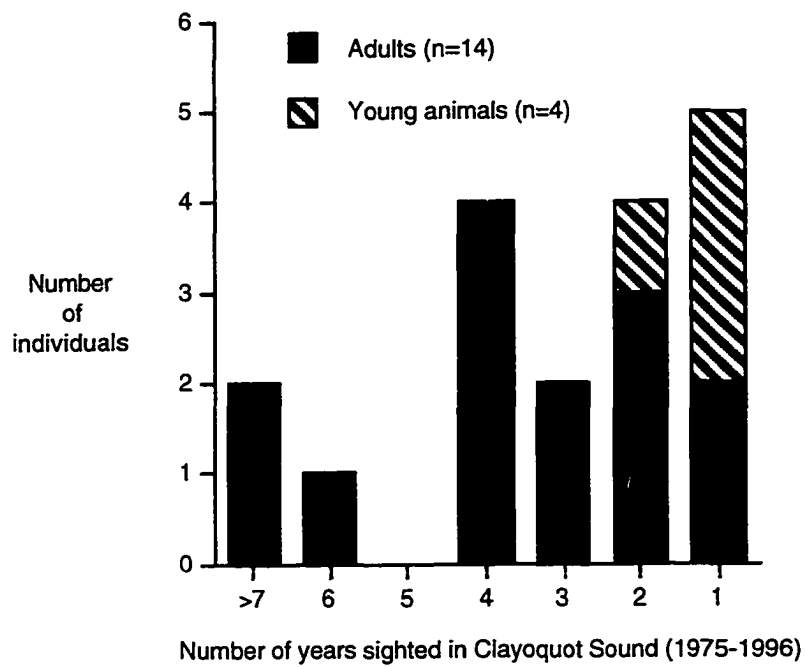
Figure 2.   Sighting history of resident gray whales from which skin biopy samples were collected.

determined by comparison of the photographs with those in a photo-identification catalog for the area (J. Darling, unpublished data).

Samples were stored in a preservative solution of supersaturated NaCl and 20% DMSO (Amos and Hoelzel 1991).

## DNA Extraction

DNA was extracted from the majority of the tissue samples using standard protocols by cell lysis in 50 mM Tris (pH 7.5), 1 mM EDTA (pH 8.0), 100 mM NaCl and 0.5% SDS and digestion with approximately 1 mg Proteinase K for a minimum of 6 hours at 55°C followed by phenol/chloroform extractions and precipitation with ethanol (Sambrook et al. 1989). Tissue samples that consisted entirely of epidermis were softened in a solution of 10 mM Tris (pH 8.0), 2 mM EDTA (pH 8.0) and 10 mM NaCl for 6 hours at 65°C and then digested with 5 mg Collagenase for 6 hours at 37°C prior to the addition of SDS and Proteinase K as described above.

## Sex Determination

The sex of each individual sample was determined by PCR amplification of homologous regions on the sex chromosomes using the three primer ZFY/ZFX system described by Bérubé and Palsbøll (1996) with one notable modification. The specificity of the AT-rich ZFY0152R reverse primer, and hence the yield of the male-specific band, was increased by the addition of TMAC (tetramethylammonium chloride) to the amplification reaction. TMAC

causes an AT base pair to be as thermally stable as a GC base pair; in low concentrations, it tends to increase the specificity and yield of PCR products amplified with AT-rich oligonucleotides (Chevet et al. 1995). Reactions were performed in 20 μL volume containing 10 mM Tris-HCl, 50 mM KCl, 60 mM TMAC, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1 unit AmpliTaq™ DNA Polymerase, 0.5 μM of each primer and 10 - 100 ng of extracted DNA. Positive and negative control reactions were performed using DNA extracted from samples obtained from animals of known sex and no DNA, respectively. The temperature profile consisted of 1 min at 94°C, 45 sec at 52°C and 30 sec at 72°C for 30 cycles preceded by a 5 min denaturing step at 92°C. PCR products were separated and visualized by 2% NuSieve™ gel electrophoresis and ethidium bromide staining. Sex was assigned on the presence or absence of the male-specific band (Bérubé and Palsbøll 1996) (Figure 3).

### PCR Amplification and mtDNA Control Region Sequencing

Symmetric double-stranded PCR amplifications of the 5' end of the mitochondrial control region were conducted using the forward and reverse primers MT4-F (Árnason et al. 1993) and Mn312-R (Palsbøll et al. 1995), respectively. Reactions were performed in 50 μL volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1 mM $MgCl_2$, 0.2 mM of each dNTP, 2.5 units AmpliTaq® DNA Polymerase, 0.5 μM of each primer and 10 - 100 ng of extracted DNA. The temperature profile consisted of 1 min at 94°C, 1 min at

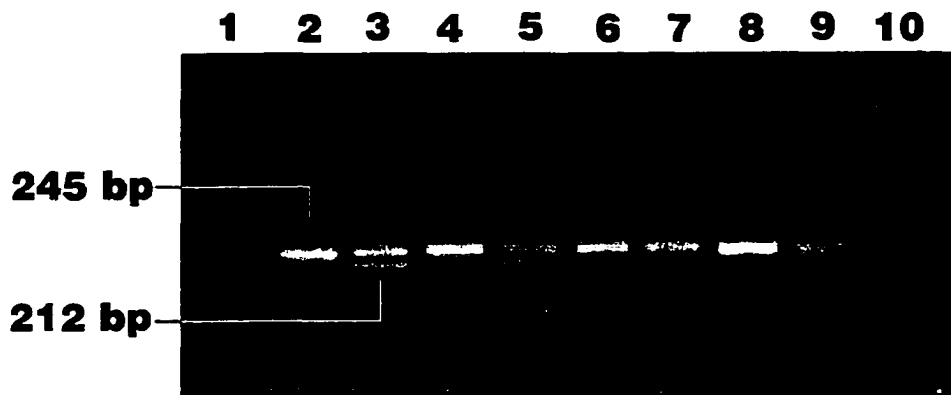Figure 3. Sex determination of gray whale samples. Lane1, negative control; lanes 2-7, samples of unknown sex; lane 8, female positve control; lane 9, male postive control; lane 10, restriction endonuclease HaeIII digested φX174 DNA.

61°C and 1 min at 72°C for 25 cycles preceded by a 5 min denaturing step at

92°C. Both the forward and reverse strands of the PCR products were

sequenced using the primers mentioned above and the ABI PRISM™ Dye

Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA

Polymerase, FS according to the manufacturer's instructions (Perkin Elmer

Corporation, Applied Biosystems Division). Sequences were scored using the

ABI PRISM™ Model 373A Sequencing System (Perkin Elmer Corporation,

Applied Biosystems Division) and aligned in Sequencher™ 3.0 (Gene Codes

Corporation).

## Data Analysis

Nucleotide and haplotype diversity was estimated as defined by Nei

(1987). Nucleotide diversity was calculated using the program NucDiversity

1.0a written by C.E. McIntosh (Molecular Genetics Laboratory, National

Zoological Park, Smithsonian Institution).

The extent of genetic differentiation between various hierarchial

partitionings of the data was estimated using the sequence ($K_{ST}$) statistic

(Hudson 1992, Hudson et al. 1992, Roff and Bentzen 1992). $K_{ST}$, an analog of

Wright's (1951) $F_{ST}$, uses the number of differences between sequences to test

whether two or more localities are genetically different and is appropriate for

data sets with high mutation rates (e.g. large number of haplotypes at low

frequencies) or small sample sizes in one or both localities (Hudson et al.

1992). Samples were considered to be homogeneous unless the probability of obtaining the observed $K_{ST}$ value was less than 0.05 in 1000 Monte Carlo simulations (Hudson et al. 1992). Pairwise homogeneity tests were conducted in the following hierarchial manner: adult vs. young residents, male vs. female residents and non-residents and residents vs. non-residents. Homogeneous partitions were pooled in subsequent comparisons.

A midpoint-rooted genealogical tree was estimated by the neighbor-joining (NJ) method (Saitou and Nei 1987) and evaluated using the bootstrap procedure (Felsenstein 1985) based on 1000 re-samplings using the test version 4.0d63 of PAUP* written by D.L. Swofford (Laboratory of Molecular Systematics, Smithsonian Institution). The pairwise genetic distance matrix was computed assuming a gamma distribution of substitution rates across nucleotide sites (Tamura and Nei 1993, Wakeley 1993). A value of 0.5 was used to estimate the alpha parameter (a) in the Tamura and Nei (1993) model. Wakeley (1993) demonstrated that this value is appropriate for sequences in control region 1. Values of a=0.2 and 0.9 were also used and were found to have minor effects on the NJ tree.

# CHAPTER 4

## RESULTS

### Sex Composition

The observed sex ratio among residents (adult and young whales combined) was 13 males and 5 females. Although this ratio of 2.6:1 was nominally biased towards males, it was not significantly different from parity ($\chi^2$=3.56, p=0.06). The observed sex ratio among residents, excluding young animals, was 10 males and 4 females. This ratio of 2.5:1 was also in favor of males, but it was not significantly different from parity ($\chi 2$=2.57, p=0.11). The sex ratio among non-residents was 6 males and 8 females. This ratio of 0.75:1 was not significantly different from parity ($\chi 2$=0.29, p=0.59).

### Sequence Analysis

The first 311 base pairs of the 5' end of the mtDNA control region were sequenced for 32 individuals. A total of 26 polymorphic sites defined 18 haplotypes (Table 1). All substitutions were transitions with the exception of one transversion and one indel. Eleven polymorphic sites were uninformative; eight of these sites, including the transversion and indel, were attributable to one unique haplotype (haplotype 18) that was markedly different from all other haplotypes. The other three uninformative sites occurred in three different haplotypes, one was unique (haplotype 9) and two were not (haplotype 2 and

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Table 1. Gray whale haplotype and gender summary. The first column corresponds to the haplotype numbers used in Figure 2. The second column represents the polymorphic sites which defined the 18 haplotypes observed in the 32 samples. The numbers above correspond to the nucleotide position of the polymorphic sites starting from the first position of the mtDNA control region. Haplotypes 1-18 refer to the consensus sequence in the second row: "."=identical position relative to the consensus sequence; ":"=indel. The third column lists the sample ID numbers with respect to haplotype. The letters denote the sampling location and the digits are an identification number: AK-Alaska; BC-British Columbia; CA-California; CS-Clayoquot Sound; OR-Oregon. Sample ID numbers that are underlined are males; those not underlined are females.

```
                          1 1 1 1 1 1 2 2 2 2 2 2 2 2 2 3 3 3
              6 8 8 8 9   0 0 0 0 2 4 6 6 7 8 8 8 9 0 0 1
HAPLOTYPE  5 6 7 7 8 9 7 1 3 4 5 3 0 7 3 4 5 0 3 7 8 9 8 6 9 0      SAMPLE ID NUMBER

consensus  T : T C T T T T C T T G T T G T A C T G T C C C T T

    1      . . . . . . . . . . C . . . . . . . . . . . . . . .     CA84260
    2      . . . . . . . . . C C . . . . . . . . . . . . . . .     CS9612, AK4849
    3      . . . . . . . . . . . . . . . . . A . . . . . . . .     CA88981, AK5574
    4      . . . . . . . C . . C . . . . . . A . . . . . . . .     CS752, CS908, CS9422
    5      . . . . . . . . . . C . . . . . . A . . . . . . . .     CS928, CA85979
    6      . . . . . . . . . . C . . . . . T . . . . . . . . .     CS9417, CS955, CA88980, AK4850, CA002
    7      . . . . C . . . . . . . . . . . T . . . . T . C . .     CS901, CS939
    8      . . . . . . . . . . . . . . . . . . . . C . . . . .     CA4885
    9      . . . . . C . . C . . . . . . . A . T . . C . . . .     CS794
   10      . . . . . . . . . . . . . . . . G . . . . T . . . .     CS9426
   11      . . . . . . . . . . . . C . . . G . C . . T . . . .     CS951, CS952, CS956, CA001
   12      . . . . . . C . . . . . . . . . G . C . . T . T . .     OR4529
   13      . . . . . . C . . . C . . . . . G . C . . T . T . .     CS9011
   14      . . . . . . . . . . . . . . . C G . C . . . . . . C     CS957
   15      . . . . . . . . . . . . . . . C G T C . . T . . . .     CA85967
   16      . . . . . . . . . . . . . . . C G T C . . T . . . C     CS9423
   17      . . . . . . . . . . . . C . . . G T C . . T . . . .     CA4836, BC001
   18      A C C T . C . . T . . A . C . C G T C . . . . T C .     CS9514
```

7). The majority (56%) of haplotypes were unique; five haplotypes were attributed to two samples and only three haplotypes occurred in more than two samples. Only four of the eight haplotypes present in two or more samples consisted exclusively of residents or non-residents (haplotype 4 and 7; haplotype 3 and 17, respectively). The average sequence divergence between all haplotypes excluding haplotype 18 was 1.7% whereas the average sequence divergence between haplotype 18 and all other haplotypes was 4.2%.

## Nucleotide and Haplotype Diversity

The estimated nucleotide diversity for the total sample (i.e. the eastern North Pacific population) was 0.017 (SE=0.002) and the haplotype diversity was 0.94 (Table 2).

## Homogeneity Tests

No heterogeneity was detected within adult vs. young residents and male vs. female residents and non-residents therefore the samples within the residents and non-residents were pooled. The subsequent comparison revealed no heterogeneity between residents and non-residents (Kst=-0.02, p=0.79).

## Estimated Genealogy

The neighbor-joining tree based on Tamura-Nei corrected distances (a=0.5) among haplotypes revealed three clades that did not correspond to any

obvious geographic pattern (Figure 4). Each clade contained haplotypes which occurred in both residents and non-residents; furthermore, only one of the four nodes supported by a bootstrap value of over 50% contained haplotypes that occurred in residents only. The very different, unique haplotype (haplotype 18) was basal to all other haplotypes reflecting the distance between it and all other haplotypes.

Table 2. Estimates of nucleotide and haplotype diversity in other mysticete populations.

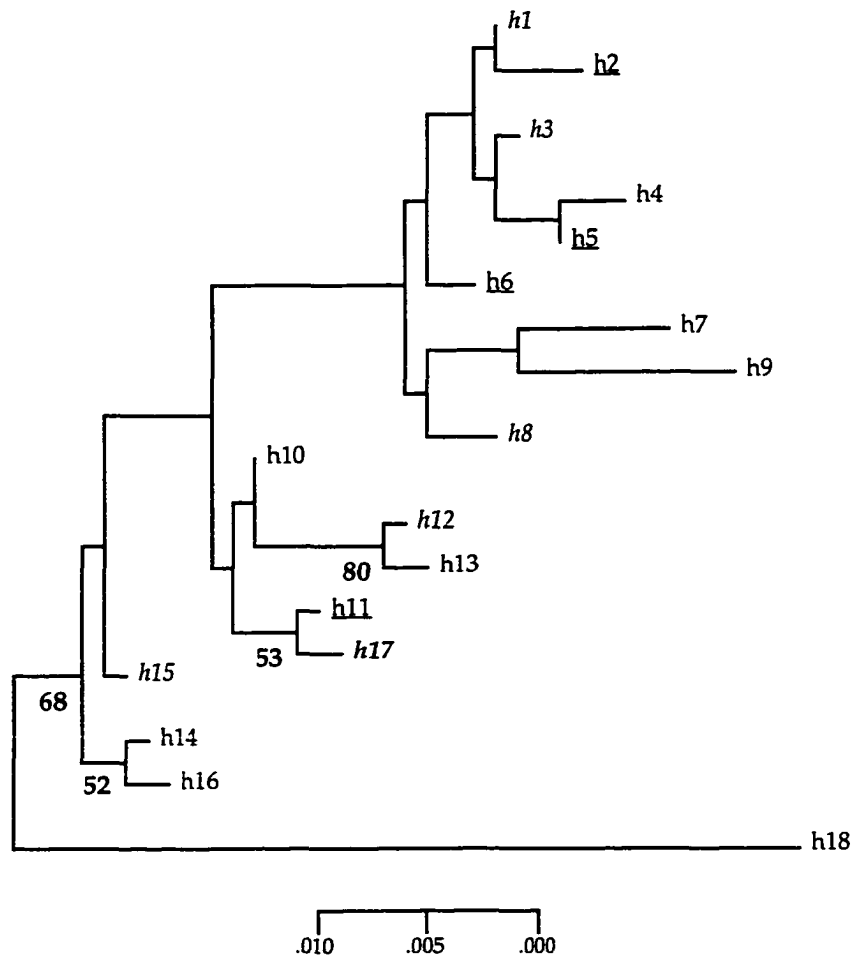| Species | mtDNA control region sequence | Nucleotide diversity | Haplotype diversity | Reference |
|---|---|---|---|---|
| *Megaptera novaeangliae* | 288 bp | | | |
| W. Greenland | n=44 | 0.026 | 0.86* | Palsbøll et al. 1995 |
| Iceland & Jan Mayen | n=85 | 0.016 | 0.64* | Larsen et al. 1996 |
| N. Norway & Bear Island | n=35 | 0.008 | 0.53* | Larsen et al. 1996 |
| | | | | |
| *Balaenoptera acutorostrata* | 345 bp | | | |
| N. Atlantic | n=87 | 0.006 | 0.86* | Bakke et al. 1996 |
| Antarctic | n=23 | 0.016 | 0.98* | Bakke et al. 1996 |
| | | | | |
| *Eubalaena australis* | 293 bp | | | |
| Argentina | n=20 | 0.021 | 0.95 | V. Portway, in preparation |
| South Africa | n=21 | 0.027 | 0.94 | V. Portway, in preparation |
| | | | | |
| *Eschrichtius robustus* | 311 bp | | | |
| E. North Pacific | n=32 | 0.017 | 0.94 | this study |
| E. North Pacific | n=32 | 0.018 | 0.93 | P. Rosel, unpublished data |
| W. North Pacific | n=9 | 0.017 | 0.50 | P. Rosel, unpublished data |

*estimated using data reported in reference

Figure 4. Neighbor-joining tree based on Tamura-Nei corrected distances (a=0.5) among haplotypes (defined in Table 1). Nodes supported in over 50% of 1000 bootstrap replications are indicated in bold below the nodes. Haplotype numbers that occur in residents only, non-residents only or both are indicated by plain, italized and underlined text, respectively.

CHAPTER 5

DISCUSSION

## Sex Composition

When studies first began, the age and sex composition of the gray

whales that summer in Clayoquot Sound were unknown (Hatler and Darling

1974). Long-term sighting studies have since demonstrated that both adults

and young animals are present in the Sound, although generally only adults

appear to exhibit fidelity to this site (Hatler and Darling 1974; Darling 1978,

1984; Darling et al. in press). This study indicates that animals of both sexes

summer in the Sound. The sex ratio among Clayoquot Sound residents was

2.6 to 1 in favor of males, but was not significantly different from parity. Further

studies based on a larger sample size are required to determine if this male

bias is indeed significant.


## Genetic Variability

The relatively high levels of nucleotide and haplotype diversity in the

eastern North Pacific gray whale population are comparable to that of other

recovering mysticete populations; the nucleotide diversity in the eastern and

western North Pacific gray whale populations are also very similar (Table 3).

The eastern North Pacific gray whale population was depleted by two periods of

whaling in the mid 19th and early 20th centuries (Gilmore 1955, Henderson

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1984). Several authors postulated that few (<50) animals remained at the end

of each whaling period (Andrews 1914, Howell and Huey 1930). However,

more recent estimates suggest that many more whales survived; approximately

5000 and 9000 animals, respectively (Ohsumi 1976, Storro-Patterson 1977).

The observed levels of genetic variation lend support to the latter scenario.

However, Amos (1996) cautioned that historical reductions in population size

will only be reflected in low levels of genetic variation if the population spent a

prolonged period of time at low numbers. This may explain why the level of

nucleotide diversity in the eastern and western North Pacific gray whale

populations are very similar despite the fact that the western North Pacific

population was severely reduced by prolonged whaling activity that began in

the late 16th century and persisted into the mid 20th century (Berzin et al. 1995,

Kato and Kasuya in press) and remains small at approximately 250 animals

(Vladimirov 1994). The low level of haplotype diversity may be more indicative

of historical reductions in this population; however, both diversity estimates for

the western North Pacific population are based on a very small sample size.

## Lack of Heterogeneity

Studies in other mysticetes have demonstrated that long-term fidelity to

summer foraging areas is determined matrilineally. For example, photo-

identification studies document the annual return of mothers and their calves to

specific feeding areas in the western North Atlantic for humpback (Katona and

Beard 1990, Clapham et al. 1993), right (Schaeff et al. 1993) and fin whales

(Clapham and Seipt 1991). The majority of humpback whale calves remain with their mothers until the end of their first year (Clapham and Mayo 1990). Right whale calves appear to be weaned sometime near the end of their first year although variability in weaning time occurs (Hamilton et al. 1995). Weaning time in fin whales is uncertain but appears to occur when calves are approximately six to eight months old (Clapham and Seipt 1991, Seipt et al. 1990). Clapham and Mayo (1987) suggested that fidelity to summer foraging areas is due to calves learning migratory routes and destinations from their mothers.

Although such fidelity may persist over many generations, it can be difficult to detect genetically. For example, although photo-identification studies in the western North Atlantic indicate that humpbacks exhibit matrilineal fidelity to several relatively discrete summer foraging areas within this region, analysis of mtDNA control region sequence data suggest that the population is genetically homogeneous (Palsbøll et al. 1995). Palsbøll et al. (1995) suggest that this discrepancy is due to life history and evolutionary time scale differences; the majority of humpback whale feeding grounds in the western North Atlantic only became accessible about 10,000 years ago after the last glaciation (Johnsen et al. 1992). Even if relatively discrete matrilineally directed feeding aggregations have been established since the retreat of the ice, a much greater time scale would be necessary for evolutionary processes (e.g. stochastic lineage sorting - Avise 1984, accumulation of significant numbers of

base substitutions - Hoelzel et al. 1991) to generate similar structuring in the mt genome. However, the finding that there is significant subdivision between humpback populations in the western, central and northeastern North Atlantic (Palsbøll et al. 1995, Larsen et al. 1996) indicate that matrilineal fidelity to summer foraging areas can persist on an evolutionary timescale in some areas such that it results in the genetic differentiation of populations.

The situation for gray whales that summer in Clayoquot Sound remains unclear. The data presented here suggest that Clayoquot Sound residents are not genetically distinct from the overall eastern North Pacific population. If this finding is borne out with future work, it would suggest that fidelity to Clayoquot Sound is either not matrilineally directed, or it has not persisted long enough to be manifest in the mt genome. Eastern North Pacific gray whales have a similar evolutionary history to that of western North Atlantic humpback whales; the Chukchi and Bering seas were likely unavailable to the population during the last Pleistocene glacial maxima (Gaskin 1982) therefore it is plausible that even if the eastern North Pacific population is segregated into relatively discrete matrilineal aggregations throughout its foraging range we will not be able to detect it in the mt genome for the reasons discussed above. Furthermore, studies in some mysticetes (e.g. right whales - Schaeff et al. 1993) indicate that males generally demonstrate less site-fidelity than females. If gray whales display similiar behavior, it is possible that even if fidelity to Clayoquot Sound is matrilineally directed and significant genetic differentiation exists, it would be

difficult to detect in this study given the small number of samples obtained from female residents.

The situation in Clayoquot Sound is further complicated by the fact that although gray whales of different ages are present, only adults appear to exhibit fidelity to this site; young animals, which may or may not arrive with their mothers, do not tend to return to Clayoquot Sound in subsequent years (Hatler and Darling 1974; Darling 1978, 1984; Darling et al. in press). In light of this, other explanations for the fidelity of whales to the Sound should be considered. For example, if the site at which a gray whale calf is weaned does not dictate where it is likely to feed as a adult, young animals may spend time foraging in different feeding areas, perhaps following older animals, before selecting a specific site that they will then return to year after year. Given that studies in humpback, right and fin whales indicate that fidelity to summer foraging areas is determined matrilineally, it may seem odd to suggest that such fidelity is absent in gray whales. However, the period of association between gray whale cows and calves appears to be shorter than that of most other mysticetes. Examination of the reproductive organs of females taken in the Chukotka subsistence harvest (Blokhin 1984) suggest that almost all gray whale calves are weaned by the beginning of July (Blokhin 1984, IWC 1993), which is approximately five months after peak calving (Rice et al. 1981). Observations of lone small (<8m), presumably very young animals in Clayoquot Sound as early as June suggest that the lactation period may be even shorter for some whales

(Darling 1978, 1984; Darling et al. in press). If a high degree of learning does indeed occur during this period as suggested by Clapham and Mayo (1987), perhaps there is not enough time for gray whale calves to learn migratory routes and destinations from their mothers.

Darling (1984) predicted the animals that summer in Clayoquot Sound are part of a larger northwestern coast population. Recent photo-identification matches support this idea; several Clayoquot Sound residents have also been identified feeding along the central mainland coast of British Columbia and off the Washington coast (J. Calambokidis, J. Darling and V. Deecke, unpublished data). Recent photo-identification studies in Neeha Bay, Washington document the return of one young animal which was sighted with its mother in the previous year (J. Calambokidis, unpublished data). This sighting suggests that matrilineally directed site-fidelity may exist to some degree.

In summary, I can say only that it does not appear that the fidelity of gray whales to Clayoquot Sound is matrilineally directed. Clarification of this issue requires a longer time series of photo-identification data, as well as a larger sample size for genetic analysis (which includes an analysis of statistical power - e.g. Taylor et al. 1997); furthermore, both data sets should be expanded to include the larger northwestern coast population.

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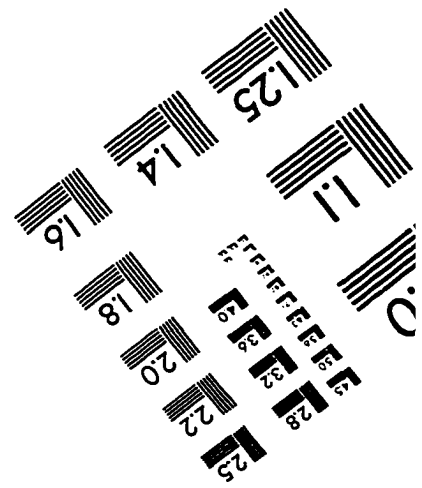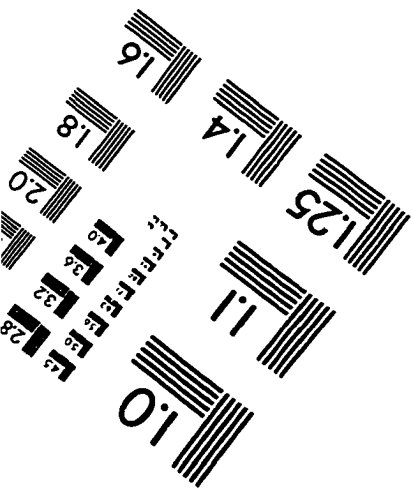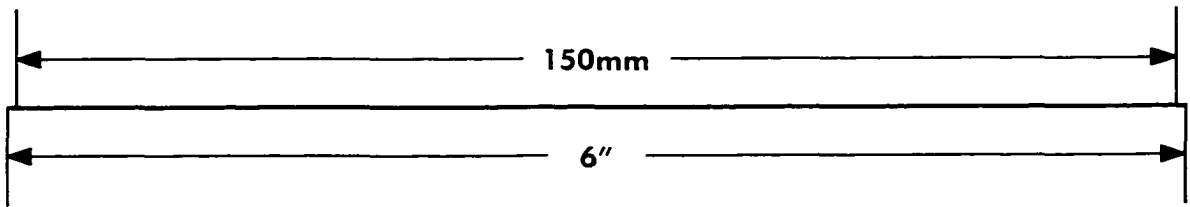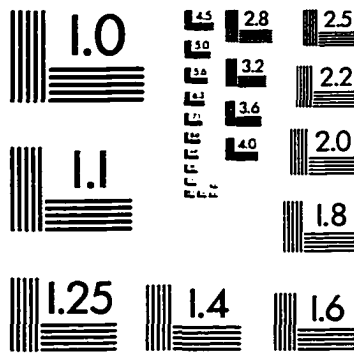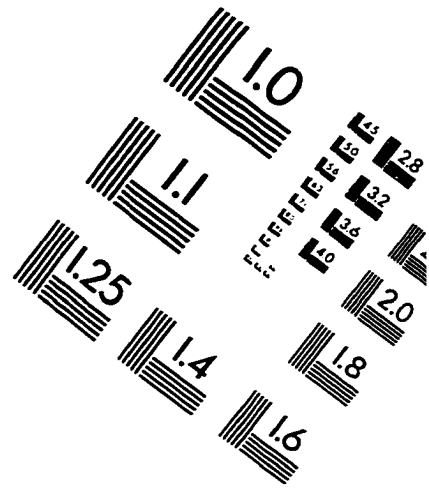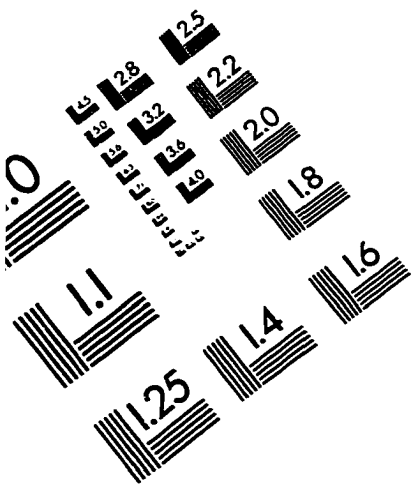
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