Exxon Valdez Oil Spill
Restoration Project Annual Report

Photographic and Acoustic Monitoring of Killer Whales in Prince William Sound and Kenai Fjords, Alaska

Restoration Project 99012
Annual Report

This annual report has been prepared for peer review as part of the Exxon Valdez Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report

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April 2000

Photographic and Acoustic Monitoring of Killer Whales

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Study History: The current project was initiated under Restoration Project 95012 (Comprehensive Killer Whale Investigations). This is the fifth annual report for this study Prior to the current years work killer whales were monitored in Prince William Sound, Alaska with funding from the *Exxon Valdez* Oil spill Trustee Council in 1989, 1990, and 1991 (Dahlheim, M.E. and C.O. Matkin, 1993) and in 1993 (Dahlheim 1994). The North Gulf Oceanic Society (NGOS) independently maintained a monitoring program in 1994. A peer reviewed 1995 annual report was submitted in April 1996 and annual reports without review comments addressed were submitted in March 1997, 1998 and 1999. An assessment of the status of killer whales from 1984 to 1992 in Prince William Sound was published (Matkin et al. 1994). Feeding habit studies, geographic information system, and genetic studies were initiated in 1995 (95012a) and continued in 1996 (96012a) and 1997 (97012a). Journal articles describing killer whale movement and distribution (Matkin et al. 1997), resident pod genealogies and status of AB pod (Matkin et al 1999a) and feeding habits (Saulitis et al 2000) have been published.

Abstract: Monitoring of killer whales (*Orcinus orca*) was continued in 1999 using photo-identification and acoustic methods. There was one calf recruited and two mortalities in AB pod. AB pod continues to show lack of recovery. While AB pod recruitment rates have averaged slightly higher than other resident pods over the last 8 years, the mortality rate has been double that of other pods. Recovery is dependent on reduced mortality rates. Disruption in social structure due to mortalities at the time of the spill is thought to be the primary cause of non-recovery.

Nine individuals missing from the AT1 transient group since 1990 and two missing since 1992 are presumed dead. There has been no recruitment in this genetically distinct population since 1984 and no recovery from losses following the spill. Lack of recovery may be a result of several factors including high levels of contaminants (PCBs and DDTs), sharp decline in harbor seals, their primary prey, and the genetic/social isolation of this group.

Improved techniques have been developed for acoustic monitoring of whales in winter months and our acoustic catalogue has been augmented by recordings made in 1999.

Key Words: acoustics, biopsy, contaminants, *Exxon Valdez*, Geographic Information System, genetics, killer whales, photo-identification, *Orcinus orca* , Prince William Sound, resident, transient.

Project Data: Identification data for individual whales consists of frame by frame identifications of individual whales for all exposed films. These identifications are available on computer disk upon request approved by the *Exxon Valdez* Oil Spill Trustee Council from Craig Matkin, North Gulf Oceanic Society (NGOS), P.O. Box 15244 Homer, Alaska (907) 235-6590. All field observations, killer whale encounter data, vessel logs and tracklines are stored in a GIS system (Arc/Info) housed at the U.S. Fish

and Wildlife Service, Marine Mammals Management, 1011 Tudor Rd, Anchorage, Alaska. Contact Doug Burn 1 800 362-5148. This data is now available for inspection and use with permission of the NGOS or U.S.FWS.

Citation: Matkin, C.O., D. Scheel, G. Ellis, L. Barrett-Lennard, H. Jurk, and E. Saulitis. 2000. Photographic and acoustic monitoring of killer whales in Prince William Sound and Kenai Fjords, Alaska (Restoration Project 99012), North Gulf Oceanic Society, Homer, Alaska.

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EXECUTIVE SUMMARY

Killer whales were monitored in Prince William Sound, Alaska with funding from the *Exxon Valdez* Oil Spill (EVOS) Trustee Council in 1989, 1990, and 1991 (damage assessment) and in 1993 (restoration monitoring). Monitoring was continued in 1995-1999 as part of the EVOS Trustee Council restoration program. The North Gulf Oceanic Society (NGOS) independently maintained a monitoring program in all other years since 1984 (Matkin et al. 1994). This report summarizes results of the monitoring of killer whales in Prince William Sound in 1999 using photo-identification and acoustic techniques. The goal of the photo-monitoring has been to obtain identification photographs of all whales in all major resident pods including AB pod and of the AT1 transient group on an annual basis. Photo-identification techniques (after Bigg et al. 1990) were used to identify individual whales. The current photographic database includes tens of thousands of frames of film collected from 1984-1999 and used to provide individual identifications for each encounter with whales. Vital rates for AB pod and all other frequently sighted resident pods were calculated based on the photographic data.

The total number of whales in well-known resident pods other than AB pod has increased from 66 to 86 whales from 1988 through 1999, while AB pod has declined from 36 whales to 24 whales in that same time period. All resident pods have increased since 1984 except AB pod. From 1995 to 1999, AB pod has had a net increase of two individuals, due to recruitment of six calves and four mortalities.. Seven members of the pod (AB 25 subpod) still appear to travel with AJ pod. Although recruitment rates for AB pod now exceed those of other pods, the mortality rate for this pod remains over twice that of other pods. AB pod is not recovering to pre spill numbers and will not until mortality rates decline.

Encounter data for the AT1 transient group (a genetically unique population) was used to update sighting histories for this group in 1999. Despite substantial field effort, the number of AT1 whales sighted each year has declined following 1989 and remains consistently half or less of what it was prior to the spill. We are confident that 11 of the original 22 whales in the AT1 group have died since the spill. The rate of encounter with members of this group also has declined significantly since 1989. Only seven of the original 22 whales attributed to the AT1 group were photographed in 1999. Listing of the AT1 group under the ESA or MMPA is being considered.

Acoustic monitoring relies on a catalogue of distinct pulsed calls for each resident pod, the AT1 group, and the Gulf of Alaska transients collected from 1984 to 1998. Distinct pod/population repertoires allow identification from recordings collected by remote hydrophones. During winter 1998-99 a remote hydrophone was operated in Resurrection Bay, however unexpected ambient noise levels and transmission problems made it essentially non-functional much of the winter. It has been replaced by a system in another location that uses microwave transmission powered by wind and solar electrical systems. This system has been used successfully and results will be reported in FY2000

Data on killer whale behavior and predation events were recorded in a standard format during all years of the monitoring program. Vessel tracks and maps of whale movements were also maintained. Data entry into the GIS database has been completed for all NGOS killer whale records from 1984 to 1999, including a total of 1,808 boat-days of search effort and 811 encounters with whales. In 1999 the GIS database was transferred to a location at Marine Mammal Management, U.S.F.W.S. Anchorage, Alaska and a copy supplied to Exxon Inc. by request under the Freedom of Information Act.

Biopsy tissues from free ranging whales were collected on an opportunistic basis from six transient whales in 1999 using a biopsy dart system developed by Barrett-Lennard et al. (1996). Due to funding constraints, genetic laboratory analysis has not yet been completed; however, adipose tissue collected from these samples was used in contaminant analysis reported in Ylitalo et al. (in prep.).

INTRODUCTION

On March 31, 1989, a week after the *Exxon Valdez* Oil spill (the spill), the AB pod of resident killer whales was observed traveling through oil sheens in western Prince William Sound, and six members of the pod were missing. In the two years following the spill, a total of 14 whales was lost, and there was no recruitment into AB pod. The rate of mortality observed in this pod after the oil spill (19% in 1989 and 21% in 1990) exceeds by a factor of 10 the rates recorded over the past 11 years for the other resident pods in Prince William Sound or over the past 20 years for 19 resident pods in British Columbia and Washington State (Balcomb et al. 1982, Bigg 1982, Olesiuk et al. 1990, Matkin et al. 1994). Since the time of the spill, the social structure within AB pod has continued to show signs of deterioration. Subgroups have traveled independently of the pod, and pod members have not consistently traveled with closest relatives. The pod has been observed less often, while prior to the spill, AB pod was the most frequently encountered resident pod in Prince William Sound (Matkin et al. 1994). Although AB pod had a net gain of two whales from a low of 22 whales in 1995, it still contains only 24 whales. There were 36 whales in AB pod in fall 1988 prior to the spill.

No individual resident whale missing during repeated encounters with its maternal group over the course of a summer season has ever returned to its pod or appeared in another pod in all the years of research in Canada and the United). Subgroups of resident pods may travel separately for a season or longer; however, this has not been observed for individuals. In a few instances missing whales have been found dead on beaches, but strandings of killer whales are infrequent events and most missing whales are never found. During 1975 to 1987 only six killer whales were found on beaches throughout the entire Gulf of Alaska (Zimmerman 1991). One explanation for the lack of stranded killer whales comes from the observations of early Soviet researchers. Killer whales that were shot for specimens were reported to sink (Zenkovich 1938).

Immigration and emigration may occur among groups of transient whales. In British Columbia, infrequently sighted transients missing from their original groups for periods ranging from several months to several years or more have been resighted swimming with other groups of transient whales (Ellis unpub. data). For this reason, transient whales missing from a particular group over only several years cannot necessarily be considered dead.

Eleven of the 22 whales from the transient AT1 group have not been observed or photo-documented for at least 8 years despite extensive field effort. While mortalities in transient groups cannot be confirmed with the same certainty as for residents, it is most likely these whales are dead or that they have emigrated from theSound. Since they have not been observed in adjacent regions, and in light of sighting records prior to the spill, it is most likely they are dead. Most of these whales (9 of 11) disappeared the year of or the year following the spill Sound (see Overall Conclusions).

The AB pod and AT1 group appear to have been injured due to the effects of the *Exxon Valdez* oil spill and neither has demonstrated a recovery. Numbers of whales in other well-documented resident pods have increased following the spill. Annual photographic monitoring has been the most effective tool in determination of the recovery status of AB pod and the AT1 group and the status of the entire Prince William Sound killer whale population (Matkin et al. 1994). This project continues using photo-identification to monitor changes in resident killer whale pods (including AB pod) and the AT1 transient group in Prince William Sound/Kenai Fjords.

Previous projects examined harbor seal predation parameters using historical killer whale sighting and behavioral data in a geographic information system (GIS) framework. Predation by killer whales may be a factor in the non-recovery of harbor seals in Prince William Sound following the *Exxon Valdez* oil spill. The decline of harbor seals may also be a factor in the non-recovery of the AT1 group of transient killer whales. At least 300

harbor seals were killed at the time of the spill and the harbor seal population does not show signs of recovery from a decline that began before the spill. Of the two types of killer whales in Prince William Sound, only one, the transients, has been observed preying on marine mammals. Observation of predation and collection of prey remains has indicated harbor seals and Dall's porpoise are the primary food items of AT1 transient killer whales, at least from April to October. These results have been incorporated into models of harbor seal population dynamics (project 064, seal trophics). Resident killer whales appear to select coho salmon from mixed schools during the July to September period (Saulitis et al. 2000) and have been observed preying on chinook salmon in the May to June period.

A geographic information system (GIS) database was designed and the data from 1984 to 1999 entered into a computer from hand-written data sheets. Sighting records provide considerable behavioral information (travel rates, duration of feeding bouts, etc.). Location of encounters and basic behavioral information (resting, feeding, traveling, etc.) are available for each sighting. It has been a goal of the GIS project to provide a systematic and easily accessible storage system for geographically-referenced data generated by this ongoing project since 1984. The system can be used to address questions of interest to restoration management, and to examine the distribution of whale groups over time in Prince William Sound. Data analysis is providing detailed demographics and spatial distributions of resident and transient killer whales (Scheel et al. in review).

Past projects have examined the separation of marine mammal-eating transient whales and fish-eating resident killer whales using behavioral data and genetic analysis. Genetic samples were obtained from 103 identifiable whales. Samples were obtained using lightweight biopsy darts (Barrett-Lennard et al. 1996). The genetic analysis used both mitochondrial DNA (mtDNA) and nuclear DNA microsattelites to separate populations and examine breeding systems. MtDNA evolves quickly, is only passed through the maternal line, and provides a faithful record of female lineages over long periods. MtDNA is considered an appropriate marker for distinguishing well-established populations. Microsatellite analysis has also provided further delineation of populations and examined male mediated breeding patterns.

Contaminant analysis has been completed on blubber tissue collected simultaneously with the genetic samples. Analysis was conducted by the National Marine Fisheries Service, Environmental Contaminant Laboratory in Seattle, Washington using a rapid high-performance liquid chromatography/photodiode array (HPLC/PDA) method. This method has proven accurate in the analysis of very small blubber tissue samples. Patterns in contaminant accumulation suggest the importance of reproductive status and genealogy in determining contaminant levels. Contaminant levels in transient killer whales were 15 to 20 times higher than in resident whales and are comparable to levels in other populations believed to have been negatively impacted by contaminants.

Killer whales can be found regularly in Alaskan waters, but only a few locations allow acoustic tracking of animals for purposes of group identification and community assessment. Ambient and anthropogenic noise in some areas precludes use of remote hydrophones and may also interfere with the whales ability to communicate or hunt and may which may cause avoidance of those areas. Some parts of Prince William Sound and Kenai Fjords, Alaska are relatively acoustically pristine areas in which tracking of killer whales by calls is possible. Since the mid-1980s, during systematic field studies of killer whales of this area we have opportunistically recorded killer whale vocalizations while identifying individuals photographically. As a result, a relatively large number of acoustic recordings exist in addition to photo-identification pictures of killer whales. Acoustic analysis supports separation of populations described by genetic analysis and demonstrates resident pod specific dialects and acoustic clans which makes possible identification and enumeration of whale pods and groups from calls collected via remote hydrophone stations.

OBJECTIVES

1. To monitor changes in AB pod, the AT1 transient group and the other major resident pods in Prince William Sound.

2. To identify individual whales photographed on a frame by frame basis and complete entry of identification data for 1999 into a photographic database.

3. To complete input of observational data for 1999 into the specially designed GIS system and transfer system to U.S. Fish and Wildlife Service, Marine Mammal Management, Anchorage, Alaska.

4. To submit for publication the results of mtDNA analysis and nuclear DNA analysis.

5. To submit for publication the results of contaminant analysis in Prince William Sound/Kenai Fjords killer whales.

6. To continue analysis of acoustic data collected from 1984-1996 and determine pod specific killer whale dialects and vocal similarities between putative clans.

7. To submit for publication the results of comparisons between killer whale acoustic dialects and genetic analysis

8. To establish a newly designed remote hydrophone system in Kenai Fjords and monitor for killer whales during winter months.

FIELD METHODOLOGY

Field work for the 1999 photo-identification study was conducted from the R.V. *Lucky Star*, 12.8 m inboard diesel powered vessel which carried a 5m outboard powered console skiff. Photo-identification was conducted from the skiff while acoustic recordings were made from the R.V. *Lucky Star*. The R.V. *Lucky Star* operated in both the Kenai Fjords and Prince William Sound region. In addition, the R.V. *Whale 2*, a 7.9m, live-aboard vessel powered by a 165 hp diesel engine with inboard/outboard drive operated primarily in the Kenai Fjords region.

N.G.O.S. biologists on the R.V.*Whale 1* (a 7.8 m light motor-sail vessel with 50hp outboard) also photographed killer whales and kept vessel logs and encounter sheets during surveys directed at humpback whale photo-identification. The daily vessel logs and killer whale encounter sheets for this vessel were included in the GIS data base and used in our analysis.

Mike Brittian and one of the authors (Matkin) also used several tourboats to obtain identification photographs in the winter/early spring season. These vessels were the deisel driven 48'*Viewfinder*, *42'Resolution*, and 60'*Sikku*. The twin deisel powered 42'Misty and 42' Mariah were used for four days in conjunction with the Youth Area Watch program in mid May.

Researchers attempted to maximize the number of contacts with each killer whale pod based on current and historical sighting information to insure sufficient photographs of

each individual within the pod. Consequently, searches were centered in areas that had produced the most encounters with killer whales in the past, unless sighting information indicated changes in whale distribution. Whales were found visually, or by listening for killer whale calls with a directional hydrophone, or by responding to VHF radio calls from other vessel operators. Regular requests for recent killer whale sightings were made on hailing Channel 16 VHF. An encounter was defined as the successful detection, approach and taking of identification photographs. Accounts of whales from other mariners (generally by VHF radio) were termed "reports". Although reports were used to select areas to be searched, all identifications were made from photographs taken during encounters. Photographs for individual identification were taken of the port side of each whale showing details of the dorsal fin and saddle patch. Photographs were taken at no less than 1/1000 sec using Ilford HP5, a high speed black and white film, exposed at 1600 ASA. A Nikon N70 autofocus camera with internal motor drive and a 300 mm f4.5 autofocus lens was used. When whales were encountered, researchers systematically moved from one subgroup (or individual) to the next keeping track of the whales photographed. If possible, individual whales were photographed several times during each encounter to insure an adequate identification photograph. Whales were followed until all whales were photographed or until weather and/or darkness made photography impractical.

A vessel log and chart of the vessel track were kept for each day the research vessels operated. Similar logs were kept for all previous study years and have been placed in a GIS format and used to estimate effort (Matkin et al. 1999b). On these logs the elapsed time and distance traveled were recorded. Vessel track was plotted. Record was made of time and location of all whale sightings and weather and sea state noted at regular intervals.

Specifics of each encounter with killer whales were recorded on standardized data forms that have been used since 1984. These forms were modified in 1995 to improve collection of data for GIS input (Matkin et al. 1996). Data recorded included date, time, duration, and location of the encounter. Rolls of film exposed and the estimated number of whales photographed also were recorded. A chart of the whales' trackline during the encounter was drawn and the distance traveled by the vessel with the whales calculated. Specific group and individual behaviors (i.e. feeding, resting, traveling, socializing, milling) were recorded by time and location when possible. Encounters with whales averaged from 3-6 hours, providing considerable behavioral information (travel rates, duration of feeding bouts, etc.).

Directed observations of feeding behavior and identification and collection of prey of killer whales were made when possible during the 1999 fieldwork. Only events that provided positive evidence of a kill were categorized as predation. Evidence included prey observed in the mouth of the whale, bits of hair or other parts, or oil slicks with bits of blubber. Incidents of harassment of potential marine mammal prey were also recorded. This included instances where evidence was not observed but a kill was suspected or when potential prey exhibited fright or flight response or other strong behavioral reaction to killer whales. Harassment was demonstrated by behaviors such as flipper slapping and lobtailing by humpback whales and fleeing behavior by small cetaceans, pinnepeds, or mustelids. When predation on fish was observed, scales from the site of fish kills were collected and later identified by species. Scales were individually mounted and identifications were made by the fish scale and aging laboratory at the Pacific Biological Station, Nanaimo, B.C. Canada. Fish scales and marine mammal remains were collected with a fine mesh net on an extendible handle (5 m. maximum extension). The pod or group of killer whales and specific individuals present at the kill or harassment incidents were recorded on the encounter data sheets.

Biopsy samples were collected on an opportunistic basis in 1999 using a pneumatic rifle and custom-designed biopsy darts (Barrett-Lennard et al. 1996). A small dart was fired from a specially outfitted rifle powered by air pressure from a.22 caliber blank cartridge. The setup is similar to that used to deliver tranquilizing drugs to terrestrial

mammals in wildlife research. A lightweight plastic and aluminum dart (approx. 10 cm long by 1.2cm dia.) was fitted with a beveled tubular sterile stainless steel tip that took a small core of skin and blubber (approximately 1.6cm long and 0.5cm dia.). The sterilized dart was fired from a range of 16-20m. The dart struck the animal in the upper back, excised a small tissue sample, bounced clear of the whale, and floated with sample contained until retrieved with long handled net.

From the biopsy samples, the epidermis, which is heavily pigmented was separated aseptically from the other layers with a scalpel soon after retrieval. The dermal sample, the source of DNA, was stored at about 4 deg C. in a sterile 1.7 ml cryovial containing 1.2 ml of an autoclaved solution of 20% DMSO and 80% sodium chloride saturated with double distilled water (Amos and Hoelzel 1991). The dermis and hypodermis were made up primarily of collagen and lipid, respectively, and were frozen at -20C in autoclaved, solvent-washed vials for contaminant analysis. Contaminant analysis was conducted by the National Marine Fisheries Service, Environmental Contaminant Laboratory in Seattle, Washington using a rapid high-performance liquid chromatography/photodiode array (HPLC/PDA) method. This method has proven accurate in the analysis of very small blubber tissue samples.

Acoustic recordings were made using an Offshore Acoustics omnidirectional hydrophone in combination with Sony Walkman professional tape recorder. The hydrophone had a flat frequency response to signals ranging from 100Hz to 25 kHz. The tape and DAT recorders showed a flat response to signals up to 15kHz.

POPULATION STATUS

Introduction

Population monitoring of killer whales in Prince William Sound and adjacent waters has occurred annually since 1984. The existence of pre-spill data made it possible to determine that resident AB pod and the AT1 transient group declined following the *Exxon Valdez* oil spill and are not recovering. This project continues using photo-identification to monitor changes in resident killer whale pods including AB pod and the AT1 transient group in Prince William Sound/Kenai.

Methods

Photographic Analysis

All photographic negatives collected during the fieldwork were examined under a Wild M5 stereo microscope at 9.6 power. Identifiable individuals in each frame were recorded. When identifications were not certain, they were not included in the analysis. Unusual wounds or other injuries were noted.

The alphanumeric code used to label each individual was based on Leatherwood et. al. (1984) and Heise et al. (1992) and has been continued in the latest catalogue of southern Alaska killer whales (Matkin et al 1999c). The first character in the code is "A" to designate Alaska, followed by a letter (A-Z) indicating the individual's pod. Individuals within the pod receive sequential numbers. For example, AB3 is the third whale designated in AB pod. New calves were identified and labeled with the next available number.

Individual identifications from each roll of film were computerized on a frame by frame basis using a specially designed data entry program. From this photographic database, the actual number of whales identified and pods of whales present for each encounter was determined and included with each encounter entered in the GIS database.

Calculation of Vital Rates

Most new calves were already present at the beginning of the field season and exact birth dates could not be determined. We followed the method of Olesiuk et al. (1990) and placed the birth of all calves in January for calculation of vital rates. Thus, birth rates could not be measured, and recruitment rates represent the survival of calves to about 0.5 years of age. The determination of mothers of new calves was based on the consistent close association of calves with an adult female. (Bigg et al. 1990, Matkin et al. 1999a).

If a whale from a resident pod is not photographed swimming alongside other members of its matrilineal group during repeated encounters over the course of the summer field season it is considered missing. If it is again missing during the repeated encounters in the following field season it is considered dead States (Bigg et al.. 1990, Matkin et al. 1994, Matkin et al. 1999a,b).

Finite annual mortality rates (MR) and reproductive rates (RR) for resident pods were calculated as follows:

where: NM = number of whales missing from a pod in a given year
NP = number of whales present in a pod at end of the previous year
NR = number of calves recruited to 0.5 years in a pod in a given year
then: Mortality rate = NM/NP and Reproductive rate = NR/NP

If the year a mortality or recruitment occurred could not be determined it was split between the possible years. A mean weighted mortality and reproductive rate for all pods for all years was determined by pooling the data. Mean mortality and recruitment rates for the period 1991-1999 were compared for AB pod and the other well-documented resident pods which include AI,AK,AE,AJ, andAN10 pods.

The sex and age class of missing whales were determined from data collected prior to their disappearance when possible. In some cases sex had been determined by viewing the ventral side of the whale. Reproductive females were identified by the presence of an offspring. Whales of adult conformation at the beginning of the study that had not calved since 1983 and were not accompanied by a juvenile(s) were considered as possibly post-reproductive. Exact ages of whales could be determined only for whales born since 1983. Juveniles born before 1984 were given approximate ages by comparing the relative size of the whale and development of saddle patch and dorsal fin in photographs from 1984. Males are readily identified at about 15 years of age as their dorsal fin grows taller and less falcate than females. At sexual maturity fin height will exceed width by at least 1.4 times (Olesiuk et. al. 1990). The fin continues to grow until physical maturity (about 21 years of age).

Sighting data for individual transient killer whales was recorded. The cumulative number of different AT1 individuals was plotted against effort (days in the field) for the 1999 season and compared with similar data averaged for 1984-89 and 1990-1995. AT1 whales that had not been resighted for 6 or more years were considered dead.

Results

The *Lucky Star* completed 14 days and the*Whale 2* completed 40 days of dedicated killer whale surveys and sampling. The*Whale 1* completed an additional 24 survey days with a primary objective of humpback whale identification. The *Viewfinder* completed 7 days, the Resolution 5 days and the Sikku 4 days of surveys in conjunction with tour operations. The Misty and Mariah completed a total of 4 survey days during the Youth Area Watch program. A grand total of 98 survey days (LOG entries) were entered in the GIS database for 1999 (Table 1). Researchers traveled approximately 8359 km over a period of 869 hours. Effort was divided between the Prince William Sound and Kenai Fjords areas (Figure 1.)

Table 1. Effort by vessels in 1999.

Vessel	#Days	Distance(km)	Time (hr)
Lucky Star	14	1279	119
Whale 1	24	2012	141
Whale 2	40	2992	230
Viewfinder	7	600	14
Resolution	5	282	5
Sikku	4	720	21
Misty/Mariah	4	474	21
Total	**98**	**8359**	**550**

Killer whales were encountered on 50 occasions in 1999 (Table 2). Researchers spent approximately 113 hours traveling 807 km with killer whales.

Table 2. Encounters with killer whales by vessel in 1999

Vessel	# encounters	Distance (km)	Time (hr)
Lucky Star	2	n/a	9.5
Whale 1	3	n/a	6.3
Whale 2	25	n/a	73.8
Viewfinder	7	n/a	7.1
Resolution	4	n/a	2.5
Sikku	4	n/a	1.7
Misty/Mariah	5	n/a	12.2
Total	**50**	**807**	**113.1**

In 1999 there were thirty-nine encounters with resident pods. There were seven encounters with the AT1 transient group, three encounters with Gulf of Alaska transients and one encounter with probable residents (Table 3).

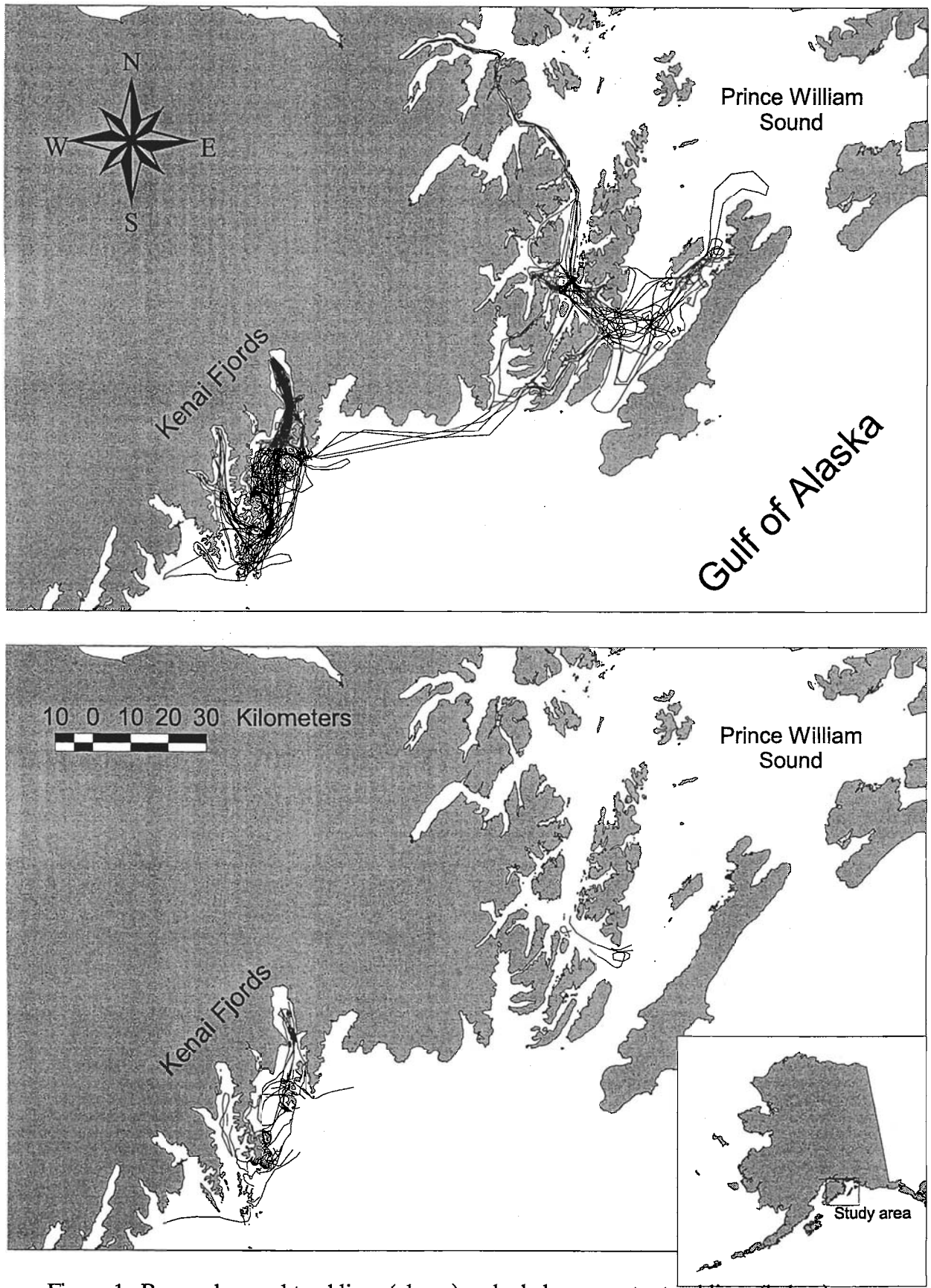


Figure 1. Research vessel tracklines (above) and whale encounter tracklines (below) for the 1999 field season in Prince William Sound and Kenai Fjords, Alaska

Table 3. Summary of 1999 encounters with killer whales in Prince William Sound (PWS) and Kenai Fjords (KF)

DAY/MO/YR	BEGIN LOCATION	END LOCATION	PODS	REGION	#WHALES
24Jan99	3.5 mi SE Seward	4.5 mi SE Seward	AB	KF	24
13Feb99	inner Res Bay	inner Res. Bay	AB	KF	24
14Feb99	1miNE Caines Hd.	1/2mi NE Caines Hd	AB	KF	24
13Mar99	Thumb Pt/Caines Hd	Thumb Pt./Caines Hd	AB	KF	24
21 Mar99	Thumb Pt.	Thumb Pt.	AB	KF	24
22Mar99	Sunny Cove	Halibut Cove,Res. Bay	AT1	KF	3
23Mar99	W. Rugged Is	W. Rugged Is	AB	KF	24
27Mar99	Thumb Pt	Thumb Pt	AB	KF	24
31 Mar99	Resurrection Bay	Resurrection Bay	AJ	KF	33
1April99	Thumb Pt	Thumb Pt.	AB	KF	24
4April 99	Thumb Pt	Thumb Pt.	AB	KF	24
5April99	N. Fox Is.	N. Fox Is.	AB	KF	24
6April99	Thumb Cove	Thumb Cove	AB	KF	24
11May99	Cape Aialik	Cape Aialik	AD16	KF	8
13May99	Cape Aialik	Cape Aialik	AD16	KF	8
14 May99	Aialik Bay, Tooth Cove	Same	AK	KF	11
15May99	W. of Hive Is	Same	AT1	KF	4
17May99	N. of Hive Is.	W. of Hive Is.	AT1	KF	3
18May99	S of Rugged I	Agnes Bay	AD05	KF	12
19May99	3miS. Shiplift	Thumb Pt.	AT1	KF	3
19May99	SE Cheval Is.	Marys Bay	AD05	KF	12
2July99	Eldorado Narrows	Porcupine Cove	AK	KF	11
3July99	Pony Cove, Res.Bay	Ciff Bay, Aialik Bay	AK	KF	11
10July99	Between Hive and Fox Is	Cape Aialik	AD16,AK	KF	19
17July99	Sunny Cove,Res Bay	Cape Res/Hive Is.	AT1	KF	3
19July99	N. End of Natoa	1.5mi SE Natoa	GOA	KF	3
27July99	Callisto Head	2mi E Bulldog Cove	AT1	KF	1
27July99	E. side Chevall Is	1mi. S. of Chevall	AD05,AD16	KF	20
27July99	E side Cheval	Mary's Bay/Chevall	AK	KF	11
28 July99	S end Cheval	1miNEChevall I.	AK	KF	11
29July99	Chat Cove	4mi. E Chevall Is	GOA	KF	3
30July99	2mi N. Harbor Is	2mi SW Holgate	AN10,AI,AK,AN20	KF	42
31 July99	No Name Is.	E Pilot Rock	AK,AI	KF	11
2Aug99	Inner Res Bay	Rugged Island	AX,AN10,20,AK,AI	KF	54
3Aug99	3miW.RuggedI.	2miWRuggedI.	AK	KF	6
6Aug99	3HoleBay,Aialik	E. Pilot Rock	AI	KF	6
10Aug99	E. Pilot Rock	N.E. Natoa	AD16,AK	KF	19
10Aug99	S end Matushka	W. Lone Rock	?	KF	5
13Aug99	1miNThumbPt.	Cape Resurrection	AT1	KF	7
14Aug99	Send Matushka	Two Arm Bay	AK,AI	KF	12
15Aug99	off Bear Cove	SW Chat Island,Aialik	AT1	KF	3
17Aug99	Aialik Cape	Natoa Island	AK	KF	6
18Aug99	Send Fox I.	3.5mi E. of Barwell I	GOA	KF	2
22Aug99	betw Rugged/Cape Res	same	AI	KF	6
22Aug99	off Agnes Bay	Nend Cheval Is.	AK	KF	6
27Aug99	Pleides/KIP	Monatague Strait	AB	PWS	18
30Aug99	Pt. Helen	Montague Strait	AB,AI,AE	PWS	40
31Aug99	Flemming I	Pleides I.	?	PWS	7
23Sept99	1mi W Fox I.	2miN ThumbPt	AB25,AJ,AN10	KF	60
26Sept99	Tonsina Cr	CainesHead/Thumb Pt	AJ,AB25	KF	41

Total Encounters: 50 Kenai Fjords: 47 PWS: 3

Despite a similar number of encounters in 1997 (50) 1998 (48) and 1999 (50), again we had fewer encounters some important resident pods such as AE and AJ pods than in 1997. There were no encounters with AG pod. The bulk of our encounters were with AB pod (in the early season), AK, AD05, and AD16 pods

Encounter rates were much lower in Prince William Sound than in Kenai Fjords again in 1999. In Kenai Fjords there were 47 killer whale encounters during 66 vessel days for an average of 0.71 encounters/day compared to an average of 0.63 and 0.79 encounters per day in 1998 and 1997 respectively. In Prince William Sound there were 3 killer whale encounters in 32 vessel days for a average of 0.09 encounters/day compared to 0.29 and 0.14 encounters per day in 1998 and 1997 respectively. The overall encounter rate for 1999 of 0.51 encounters per day was comparable to 0.49 encounters per day for 1998; however, in 1999 several smaller pods (AK,AD05,AD16) were encountered repeatedly while many larger pods were not encountered. In 1999 we had only 3 encounters with three or more resident pods ("superpods") from late July to September (Table 3) which is far fewer than in many previous years (ie. 9 superpod encounters in 1997). It is during these superpod encounters that less frequently observed pods are generally photographed. Encounters with transient whales were scattered throughout the season; however, there were no encounters with the AT1 transient group after 15 August in 1999.

Resident pods

The total number of whales in well-known resident pods other than AB pod increased from 66 to 86 whales from 1988 through 1999, while AB pod declined from 36 whales to 24 whales in that same time period (Figure 2). All resident pods have increased or are at the same numbers as in 1984 except AB pod (Figure 3).

From 1995 to 1998, AB pod showed a net increase of three individuals, due to recruitment of five calves and two mortalities. In 1999 AB pod decreased to 24 whales due to two mortalities and the recruitment of one calf. The new calf, AB55 was the first calf of AB39. The maturation of another female in AB pod brings to 9 the total number of reproductive females in the pod (Figure 4.), a number comparable to the 10 reproductive females found in the 33 member AJ pod. The mortalities in AB pod (to be confirmed in 2000) were an older male, AB5, estimated to be at least 36 years of age and AB52, the yearling calf of AB33.

Table 4 . Average recruitment and mortality rates (in percent) for AB pod and other pods from 1991-1999.

	Recruitment rate	Mortality rate
AB Pod	5.2	4.5
Other PWS Pods	4.9	2.0
B.C. Residents*	4.4	1.8

*Olesiuk et al 1990, for B.C. Northern residents 1981-1986

Recruitment has been slightly higher in AB pod than in the other pods during the post-spill period, however the mortality rate for AB pod has been more than double the rate in other pods (Table 4). Mortality rates observed in British Columbia pods (Olesiuk et al 1990) are similar to rates observed in the other pods in Prince William Sound/Kenai Fjords (Table 4).

Figure 2. Number of whales in AB pod and in all other well-documented resident pods, 1984-1999

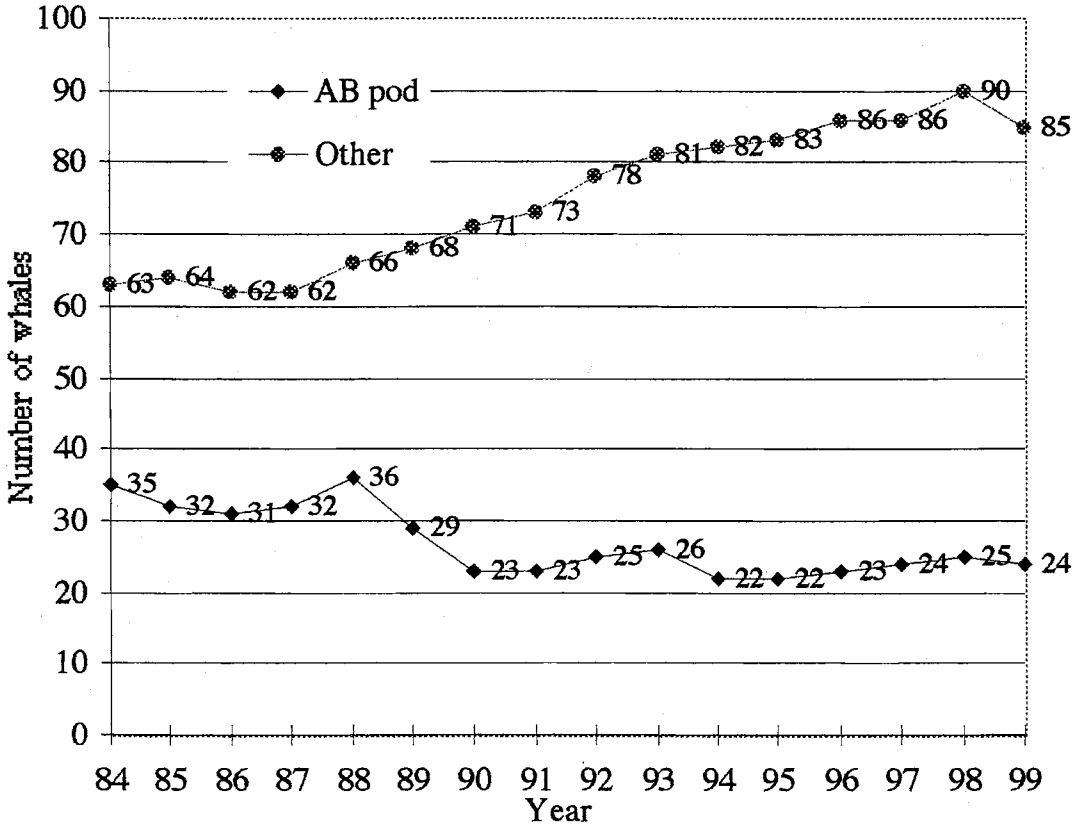
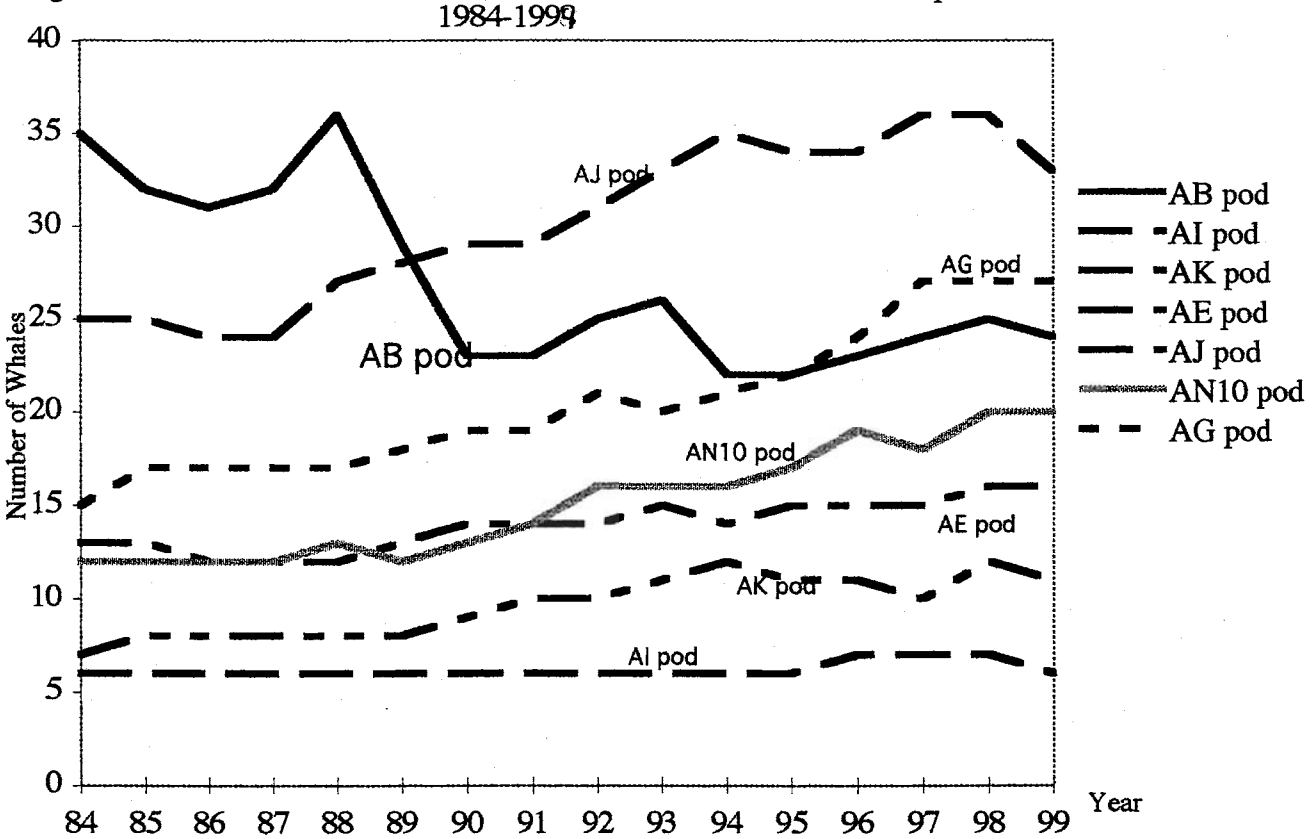


Figure 3. Numbers of whales in well-documented resident killer whale pods 1984-1999



AB Pod
AB10 Subpod

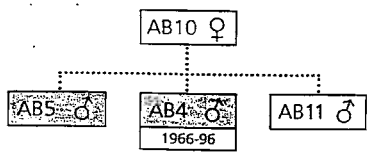
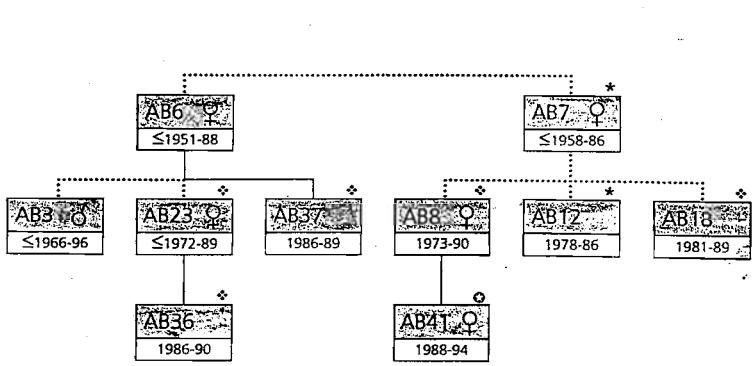
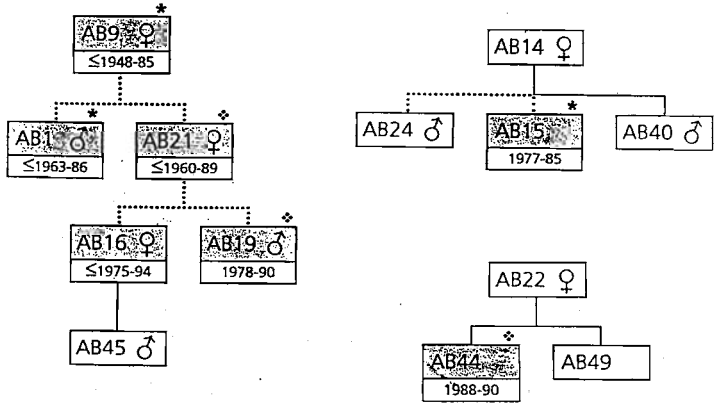
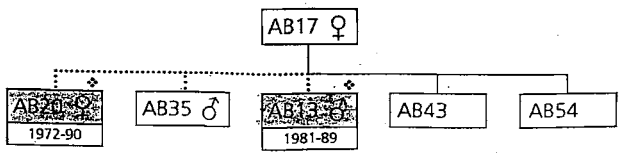


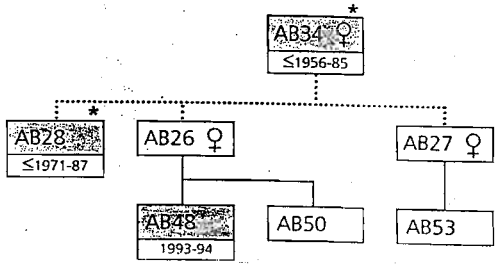
Figure 4. Matrilineal groups in AB pod illustrating the fate of individuals following the *Exxon Valdez* Oil Spill

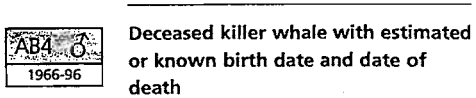
AB Pod
AB17 Subpod



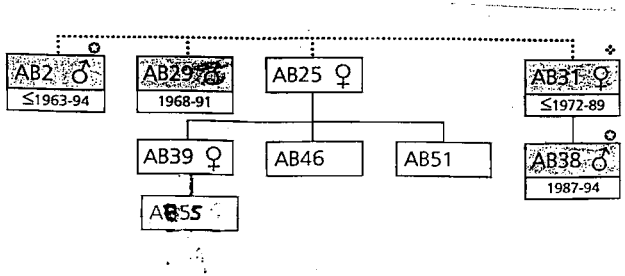


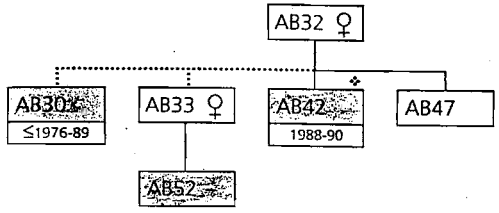






AB Pod
AB25 Subpod





AB pod was encountered on 14 occasions in 1999. The first encounter with the pod was 13 February 99 and nine of the encounters occurred before 7 April 99. Sighting reports indicated that killer whales, very possibly AB pod, were in Resurrection Bay on a regular basis throughout the January to early April period. AB pod was not present during most of the summer field season. From fieldwork, sighting reports, and data from the remote hydrophone, it appeared that AB pod was seldom in Resurrection Bay in fall 1999.

The AB25 subpod was still traveling with AJ pod in 1999, although on one occasion, on 5 April, they appeared to be traveling with AB pod without AJ pod present. During the three encounters with AJ pod in 1999, AB25 subpod was present in all encounters while the rest of AB pod was absent.

Only one calf was recruited into the other well-known resident pods in 1998/99 (Table 5). This was AJ43 born to AJ22 . There were a surprising number of mortalities in these pods; mortalities having exceeded recruitment for the first time since the 1980's. There were 5 apparent mortalities in AJ pod, including AJ 9, a 23 year old male; AJ12 and 16, a 46+ year old female and her 31 year old son; AJ 17, a 38+ year old female; and AJ18, a 33 year old male. Because AJ pod was only encountered on 3 occasions, these mortalities must be confirmed in 2000. This was the first year since 1986 that we observed a decline in the overall number of killer whales in AJ pod. There was one mortality in AK pod, the 55+ year old matriarch AK3, and one mortality in AI pod, the 34+ year old male AI1. Annual mortality and recruitment rates were calculated by pod and are listed in Table 6.

We encountered members of 10 different resident pods in 1999 (Table 7) and photographed a total of 142 resident killer whales. Pods that were completely photographed in 1999 included AB, AD16, AD05, AJ, AI, AK, and AN10. Also, three of the four matrilines that compose AX pod were photographed. AE pod was not completely documented and AG, AS, and AY pods were not encountered.

Table 7. Resident pods: number of whales and number of encounters in 1999.

Pod	#Whales	#Encounters
AB	24	14
AJ	33	4
AN10	20	3
AN20	4^	1*
AI	6	6
AE	16	1*
AK	11	13
AD16	6	6
AD5	12	6
AX	22	1**

^ only 4 whales photographed, 31 individuals previously attributed to this pod
* pod not completely photographed
** only part of pod present.

Transient whales

A total of seven of the original 22 whales from the genetically unique AT1 group were photographed during 8 encounters in 1999. These individuals were AT2, AT3, AT4, AT6, AT9, AT10, and AT18. The surviving whales that were not photographed were males pairs AT1 and AT14, and AT13 and AT17. They were photographed in 1998 but not in 1999. In recent years not all the surviving AT1 whales have been photographed in

Table 5. Recruitment and mortalities in Prince William Sound resident pods.

	Recruitment in Prince William Sound Resident Pod {whale number(mothers number)}						
POD	AB	AI	AK	AE	AJ	AN10	AG
YEAR 85			8(6)	13(11)			18(8),19(11)
86	36(23),37(6)		9(2)				
87	38(31),39(25)					38(10)	
88	40(14), 41(8) 42(32)			15(10)	26(22),27(20)	40(35)	16(11)
89	43(17), 44(22)				28(24)		17(5)
90			10(2)	18(11)	30(3)		20(4),21(10)
91	45(16)		11(6)			45(35)	
92	46(25),47(32)				31(24),32(22) 33(13	46(10),47(11)	22(5),23(15)
93	48(26)		12(7)	19(11)	34(3),35(8), 36(4)		24(11)
94	49(22)		13(2)		37(18),38(20)	48(8)	25(8)
95				20(2)		49(11)	26(6)
96	50(26),51(25)	7(4)			39(13)	50(35),51(12)	27(15),28(5)
97	52(33), 53(27)				40(3), 41(4)	54(10)	29(14),30,(11),31(10
98	54(17)	8(3)	14(7),15(9	21(5)		55(8),56(11)	
99	55(39)				42(24),43(22)		
	Mortalities in Prince William Sound Resident Pods					{by whale number}	
POD	AB	AI	AK	AE	AJ	AN10	AG
YEAR 85	9,15,34			8-			
86	1,7,12		5-	4-	23-		
87	28-					6-	
88	6-			7-			1-
89	13,18,21,23,30,31,37			12-		2-	
90	8,19,20,36,42,44						9-
91	29-						
92							
93					5-	5-	7,16
94	2,16,38,41,48			13-	11-		
95			4-				
96	4-						
97	3-		11-			49-	
98		8-			6-		
99	5*,55*	1*	3*		9*,12*,16*17*18*		
	*to be confirmed in 2000						

Table 6. Mortality and recruitment rates in Prince William Sound resident pods.

	Recruitment rates in Prince William Sound Resident Pods							
	AB	**AI**	**AK**	**AE**	**AJ**	**AN10**	**AG**	**All other than AB,AG**
85	**0**	0	14.3	7.7	0	0	13.3	3.2
86	**6.3**	0	12.5	0	0	0	0	1.6
87	**6.4**	0	0	0	0	8.3	0	1.6
88	**15.6**	0	0	8.3	12.5	8.3	5.9	8.1
89	**0**	0	0	15.4	3.7	7.7	5.9	4.5
90	**0**	0	12.5	7.7	3.4	0	11.1	4.4
91	**4.3**	0	11.1	0	0	7.7	0	2.8
92	**8.7**	0	0	0	10.3	14.3	10.5	6.8
93	**4**	0	10	7.1	9.4	0	4.8	6.8
94	**3.8**	0	9.1	0	5.9	6.7	5	4.9
95	**0**	0	0	7.1	0	6.3	4.8	2.4
96	**9.1**	16.7	0	0	0	11.8	9.1	7.9
97	**8.6**	0	0	0	5.9	5.2	12.5	3.4
98	**4.2**	14.3	20	6.7	0*	10.5	0	6.8
99	**4**	0	0	0*	5.5	0	0*	2.2
	Mortality rates in Prince William Sound Resident Pods							
	AB	**AI**	**AK**	**AE**	**AJ**	**AN10**	**AG**	**All other than AB,AG**
85	**8.6**	0	0	7.7	0	0	0	1.6
86	**9.4**	0	12.5	7.7	4	0	0	4.7
87	**3.2**	0	0	0	0	8.3	0	1.6
88	**3.18**	0	0	8.3	0	0	5.9	1.6
89	**19.4**	0	0	8.3	0	7.7	0	3
90	**20.7**	0	0	0	0	0	5.6	0
91	**4.31**	0	0	0	0	0	0	0
92	**0**	0	0	0	3.4	0	0	0
93	**0**	0	0	0	0	6.3	9.5	2.5
94	**19.2**	0	0	6.7	0	0	0	2.4
95	**0**	0	8.3	0	0	0	0	2.4
96	**4.5**	0	0	0	0	0	0	0
97	**4.3**	0	9	0	0	5.2	0	2.3
98	**0**	14.3	0	0	2.8	0	0	1.2
99	**8**	14.3	8.3	0*	13.8	0	0*	7.7
# in pod84/98	[35/24]	[6/6]	[7/11]	[13/16]	[25/33]	[12/20]	[15/27]	[63/86]

every year, and we do not suspect that these 4 males are dead. However, eleven whales in the AT1 group have been missing for eight years or more and are considered dead (Table 8, Figure 5). Since 1989, the number of AT1 individuals identified annually has been 12 or less despite a field effort that exceeded 200 vessel days in 1990 and totaled 120 days in 1997, 98 days in 1998, and 98 days in 1999 (Figure 6). There were no new calves identified in the AT1 group in 1999 and there has been no recruitment observed in this group since 1984.

The average number of different AT1 individuals sighted per field day of effort for 1990-1997 was considerably lower than for 1984-1989. In 1999 the individuals sighted per effort was below the average for both these periods. This pattern has been consistent over the past several years.

Both before and after 1989 there was an initial high rate of encounter with non-photographed AT1 individuals in the first 20 to 60 days of the field season followed by a sharp reduction of new whale discoveries despite repeated encounters with AT1 whales. This was the pattern again in 1999, when there were no sightings of new individuals after the first 20 days of the field season (Figure 6).

Five other non-AT1 transients made brief appearances in the study area. The transient whales AT109,111, and 112 (see Matkin et al 1999c) were encountered on 19July and 29July in Kenai Fjords. The two males AT30 and AT32 were encountered on 18 August in Kenai Fjords.

Discussion

There was a net loss of one individual from AB pod in 1999, and there is still no clear trend toward recovery of this pod to pre-spill numbers. It is surprising that 10 years after the oil spill under environmental conditions that seem favorable for all other resident killer whale pods, a trend toward recovery has not occurred in AB pod. Previously, we have attributed this lack of recovery to the changes in social structure and reduction in the number of reproductive females in the pod following the oil spill (Matkin et al. 1994, Matkin et al. 1999b) However, with the addition of reproductive females AB27 and AB33, who bore their first calves in 1997, and AB39, who produced her first calf in 1999, there are now nine reproductive females (whales that have produced calves in the past ten years) in AB pod, although we suspect that two of these whales, AB14 and AB17 may be near the end of their reproductive lives. Since 1991 the average annual recruitment rate for AB pod (.052) is actually slightly higher than for the other well-known resident pods (.049).

Although AB pod has exhibited above average recruitment rates in recent years (Table 1)., mortality rates continue to be over double those for other pods. The potential for recovery of AB pod is clearly dependent on a decline in mortality rate. This high mortality rate may be due to lingering physical effects of oil exposure or due to reduced fitness of individuals because of loss of close relatives at the time of the spill.

Continuing changes in social structure following the spill may play a significant role in the high mortality rates. This may be the case not only for orphaned calves or juveniles, but for other individuals in these tightly knit social units. The death of mature males following the death of their mothers is relatively common in British Columbia (G. Ellis, pers obs.). Since the oil spill, AB pod has completely lost matrilineal groups linked by the apparent sisters AB6 and AB7. This was the largest matrilineal grouping in the pod prior to the spill. Also the AB9 matriline has lost all its members except a single male juvenile, AB45. There are only two older, reproductive females, AB17 and AB14 in the AB17 subpod and no post-reproductive matriarchs. The other matrilines in AB pod are headed by younger females. This may underscore the importance of the post-reproductive matriarchs in maintenance of pod cohesiveness and in providing an environment conducive to reduced mortality rates. Although we have anticipated the stabilization and slow recovery of AB pod since the oil spill, continuing mortalities have prevented it.

Table 8. Sighting histories for all AT1 transient whales for years with effort greater than 40 days.

YEAR	AT01	AT02	AT03	AT04	*AT05*	AT06	*AT07*	*AT08*	AT09	AT10	*AT11*	*AT12*	AT13	AT14	*AT15*	*AT16*	AT17	AT18	*AT19*	*AT20*	*AT21*	*AT22*
84	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
85	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
86	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
88	X	X	X	X				X	X	X	X	X	X	X	X		X	X		X	X	X
89	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	-	-	X
90	X	X	X	X	-	X	-	-	X	X	X	X	X	X	-	-	X	X	0	-	-	-
91	X	X	X	X	-	X	-	-	X	X	-	X		X	-	-		X	0	-	-	-
92	X	X	X	X	-	X	-	-	X	X	-	-	X	X	-	-	X	X	0	-	-	-
93		X	X	X	-	X	-	-	X	X	-	-			-	-	X	X	0	-	-	-
94	X				-		-	-	X	X	-	-		X	-	-		X	0	-	-	-
95	X	X	X	X	-	X	-	-	X	X	-	-	X	X	-	-	X	X	0	-	-	-
96	X	X	X	X	-	X	-	-	X	X	-	-		X	-	-		X	0	-	-	-
97	X	X	X	X	-		-	-			-	-	X		-	-	X		0	-	-	-
98	X				-	X	-	-	X	X	-	-	X	X	-	-	X	X	0	-	-	-
99		X	X	X	-	X	-	-	X	X	-	-			-	-		X	0	-	-	-

X whale present
- whale missing, believed dead
0 whale known dead

Figure 5. Number of whales in the AT1 group based on long term sighting data.

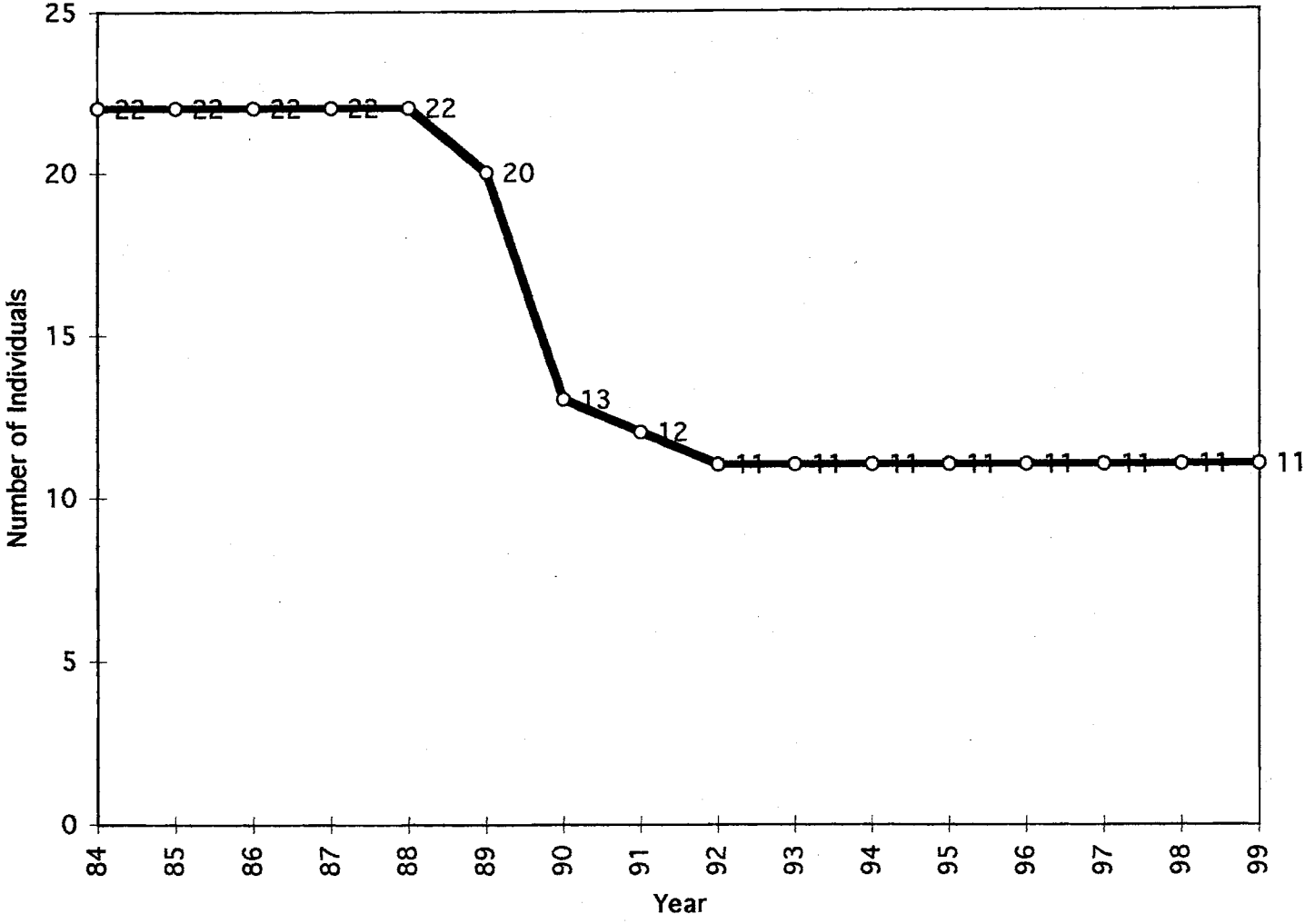
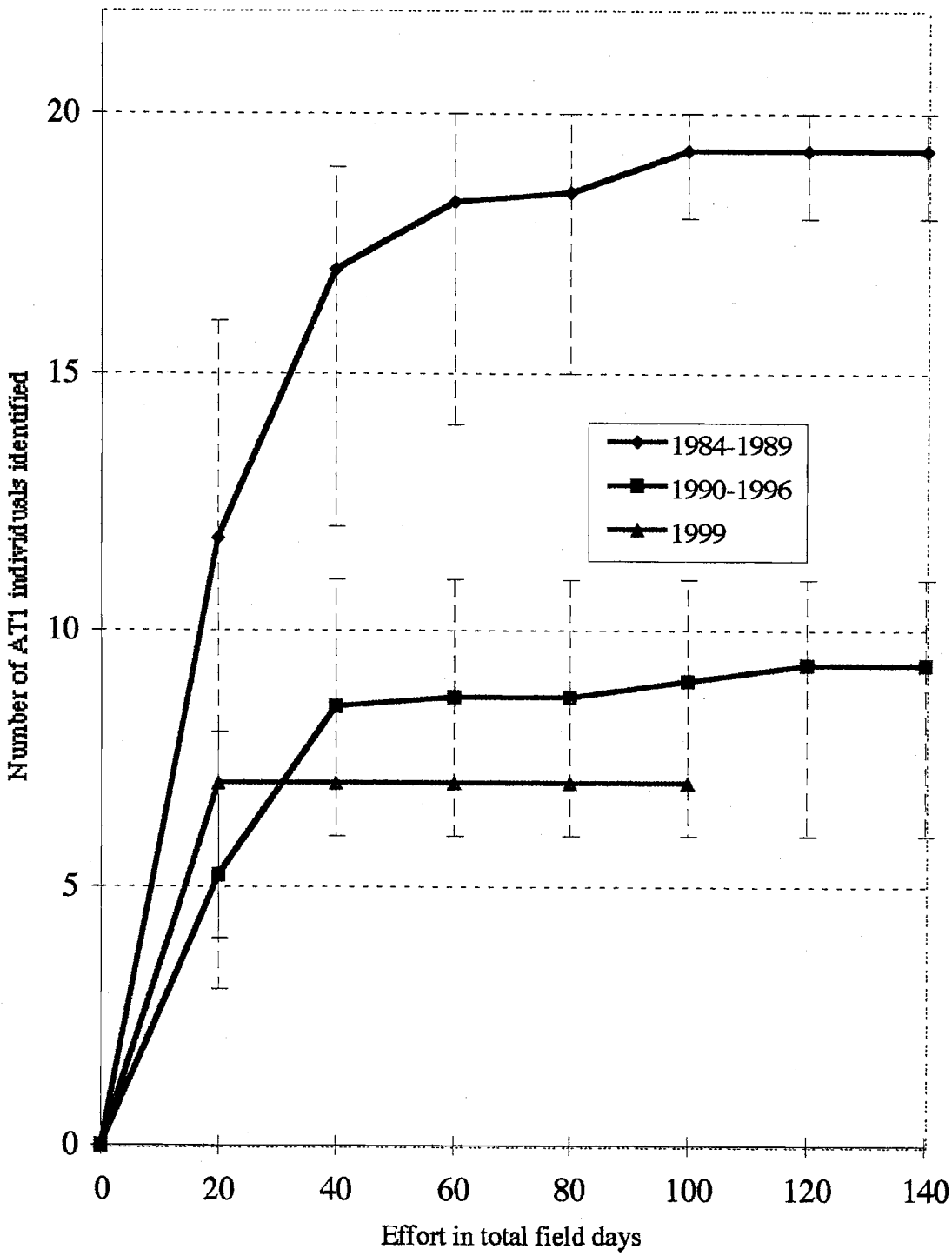


Figure 6. Average number of AT1 transient group whales identified for years with effort greater than 60 field days (error bars = range)



Although AB pod was observed as a single unit without AJ pod present on one occasion, in all other encounters it appeared that the AB25 subpod continued to travel with AJ pod. There is no precedent for a resident pod subgroup joining another pod on an extended basis (Matkin et al 1999 Bigg et al. 1990). Again, this may be a result of the breakdown of social bonds that held the subgroups within AB pod together prior to the oil spill.

AB pod appeared to be using the Resurrection Bay/Kenai Fjords on a regular basis in late winter and early spring 1999. However, they were absent during the summer months and made only irregular visits in the fall and early winter 1999. Although tourboat traffic was regular, killer whales were only irregularly sighted by operators in September and October 1999. Regular fall and early winter use of this area by resident killer whales, including AB pod, had been the pattern of the previous several years.

We are increasingly certain that 11 of the original 22 whales in AT1 transient group are now dead, nine of these individuals having disappeared since the EVOS in 1989. There have been no sightings of the missing whales in other regions since their disappearance and emigration seems highly unlikely. Also, there has been no observed recruitment into the AT1 group since 1984. This group has been determined genetically distinct by mtDNA and nuclear microsatellite DNA analysis and is acoustically distinct from all other pods and groups sampled (Saulitis et al, in prep).

The surviving members of the AT1 group are seen less frequently than in pre-oil spill years and we suspect they now are forced to range more widely in search of prey because of the severe reduction in harbor seal numbers in the region. Although we no longer observe and photograph all of the remaining 11 whales in a given year, we have not received photographs of these whales from adjacent areas. While we did not observe AT1,14,13 or 17 in 1999, there were infrequent sightings of AT1 males traveling as pairs by tourboat operators in Prince William Sound.

In 1999 Kenai Fjords had a much higher rate of encounter with killer whales than Prince William Sound, following the pattern first observed in 1997. There was considerably less research effort in Prince William Sound than in earlier years of the study. In part the high sighting rate in Kenai Fjords is due to the efficiency of the observer network made possible by the operation of numerous tourboats. Encounters in the early season were made from tourboats ("platforms of opportunity") in response to sightings by tourboats. However, it is clear from our observations as well as those of tourboats from both regions that the number of animals in the Sound has declined in recent years, particularly in the August/September period (Matkin et al. 1999b). Interviews with tour and charter boat operators suggested that few large groups (multi-pod associations) of killer whales used southwestern Prince William Sound in 1999. Photographs from these operators provided evidence of infrequent encounters with AE and AK pods in the northwestern and southwestern regions of the Sound.

We were able to photographically document the presence of AB pod (and occasionally AJ pod) using "platforms of opportunity" from January through April 1999 (Figure 3.), strong evidence that these pods are year round residents of the region and rely on nearshore habitat at all times of year. During winter killer whales often seemed to be associated with herring. We suspect they feed either on herring, or, more likely, on the chinook salmon (*Oncorhyncus twatshwa*) that also follow the herring schools and are caught regularly by winter charter boats in the Resurrection Bay.

Killer whales were observed on an almost daily basis in May and early June by tourboat operators in Kenai Fjords and during our brief field operations in May. In May the whales were feeding extensively on king salmon as evidenced by scale samples obtained from kill sites. However, many of the pods we expected to see in Kenai Fjords in late summer and fall (eg. AB, AJ, AN10, AX) only made brief appearances in 1999, unlike the previous three years when they spent extended periods in the region. This may have been in part due to the poor return of coho salmon to Resurrection Bay following years of excellent returns. The residents are known to feed on coho salmon (*Oncorhyncus*

kisutch) at this time (Saulitis 2000). The AF and AG pods that center their range in southeastern Alaska were not observed this season. It is possible that large social aggregations of killer were feeding on more abundant coho salmon in other coastal areas or offshore.

GIS DATABASE

Vessel logs and killer whale encounter sheets were entered into the GIS data base which has been transferred to the U.S. Fish and Wildlife Service, Marine Mammal Management in Anchorage, Alaska. No analysis other than data summaries and mapping were performed on the data in 1999. The annually updated GIS database will serve as an important long-term baseline in the event of future perturbations in the environment and against which changes in distribution can be assessed. The database is now opened for use by other agencies (ie. USFWS and NMFS). A copy of the entire database was provided to Exxon Inc. in response to their request filed under the Freedom of Information Act.

POPULATION GENETICS

In 1999 the principal focus of our molecular work was the summary and statistical analysis of results, and preparation and submission of journal papers . **Attached Appendix 1 is the manuscript "Reproductive isolation between sympatric and parapatric populations of killer whales in British Columbia and Alaska." submitted to the Proceedings of the Royal Society, London B.**

Although we opportunistically sampled individuals important to our long-term genetics program in 1999, there was no funding for directed genetics field sampling or laboratory analysis. We did obtain 6 samples from additional transient whales, two from AT1 transients, 2 from Gulf of Alaska transients, and 2 from Southeast Alaska/British Columbia transients. One additional sample was obtained from a resident whale (AJ41) to replace a damaged sample of the same whale taken previously. These samples are stored pending funding for genetic analysis; however, contaminant analysis was conducted on the adipose tissue from each sample and results included in a manuscript (Ylitalo et. al. in prep.) A total of 103 skin samples have been obtained by biopsy dart from unique identifiable killer whales in the Prince William Sound/Kenai Fjords region since this program began in 1994.

Some of major population level conclusions that have resulted from our genetics investigations include the following:

1.) Resident and transient killer whale lineages are reproductively isolated

2.) The resident and transient populations are divided into genetically differentiated regional subpopulations.

3.) The AT1 subpopulation is distinct from all other resident and transient groups and has higher genetic diversity than would be expected from a small remnant population.

4.) The Southern Alaska residents that occur in Prince William Sound/Kenai Fjords are a distinct subpopulation that contains two distinct mtDNA haplotypes.

5.) Fish eating and mammal-eating killer whale assemblages diverged once in the northeastern Pacific.

6.) Mitochondrial and microsatellite DNA diversity is higher in transients than residents.

7.) Resident females remain within their natal groups.

The attached manuscript (Appendix 1) further describes the genetics of eastern North Pacific killer whales. The results of these investigations have important long-term management implications for eastern North Pacific killer whale stocks and have led to investigation of potential listing of the southern resident population and AT1 transient population under the Endangered Species Act or Marine Mammal Protection Act.

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ACOUSTICS

A remote hydrophone system with permanently fixed steel shielded cable was installed off the south point of Halibut Cove, Fox Island in Resurrection Bay in summer 1998 and the acoustic feed from that station was broadcast on an FM frequency that could be monitored by researchers as well as tourboats. The signal also could be monitored in Seward, Alaska. Background and vessel noise was occasionally a problem during the summer months; however, in the fall and winter months, noise generated by swells and surf on cobble beaches several miles away became a serious problem. This rendered the hydrophone ineffective for much of the winter season and led to the installation of a new hydrophone coupled with a wind and solar powered microwave transmission system on Thumb Point, Resurrection Bay in fall 1999. This has proven a much more effective location and the new transmission system provides a more reliable and higher quality signal. The new system was monitored in Seward during the fall/winter 1999/2000 and will be reported on in the FY2000 annual report.

Calls collected during the 1999 field season included important sequences from AD05, AD16, and AJ pods. The new recordings allowed clear separation of the AD05 and AD16 pods and indicated their relationships to other whales in the AD acoustic clan. Acoustics now corroborates our findings from association analysis (Matkin et al 1999) that AD16 pod is most closely related to AK pod and may have split from that pod fairly recently. New calls were analyzed and data incorporated into the journal manuscript attached as **Appendix 2 "Parallel cultural and genetic lineages in resident type killer whales off the coast of Southern Alaska"**

Some of the major conclusions from our acoustic analysis include:

1.) The distinctive dialects identified for resident pods are apparently transmitted by learning within the maternal group, are relatively stable over time, and probably function in assortive mating as part of a system to avoid inbreeding.

2.) Two acoustic clans of resident killer whales have been identified in southern Alaska, AB clan and AD clan, and the acoustic separation is reflected in the distinct mitochondrial DNA haplotypes exhibited by each clan. AB clan shares its haplotype with the British

Columbia northern resident killer whale population and AD clan shares its haplotype with the Puget Sound/British Columbia southern resident population

3.) Dialects of resident killer whales may be culturally selected traditions that reduces competition between clans by monopolizing times and areas of increased food abundance.

4.)Calls from remote hydrophone installations can be used to determine acoustic clan and pod membership of recorded whales during the winter months.

OVERALL CONCLUSIONS

There were two mortalities in AB pod in 1999 and one calf was recruited for a net decrease of one individual in the pod. The pod currently numbers 24 whales and has shown a net gain of only two individuals since a low of 22 whales in 1995. With the addition of reproductive females AB27 and AB33, who bore their first calves in 1997, and AB39, who produced her first calf in 1999 there are now nine reproductive females (whales that have produced calves in the past ten years) in AB pod, although we suspect that two of these whales, AB14 and AB17, may be near the end of their reproductive lives. Since 1991 the average annual recruitment rate for AB pod (.052) is actually slightly higher than for the other well-known resident pods (.049). Although AB pod has exhibited above average recruitment rates in recent years, mortality rates continue to be over double those for other pods. The potential for recovery of AB pod is clearly dependent on a decline in mortality rate. The high mortality rate may be due to lingering physical effects of oil exposure or due to reduced fitness of individuals because of loss of close relatives at the time of the spill. Changes in social structure due to mortalities following the spill may play a significant role in the high mortality rates.

A total of 11 of the 22 original members of the AT1 transient group have been missing for 8 years or more and are presumed dead. There has been no recruitment within the group since 1984. Although only seven of the original 22 whales from the genetically unique group were photographed during 8 encounters in 1999, we are not certain that any additional whales have died at this time. The factors contributing to the decline of the AT1 group and its reduced role in the Prince William Sound ecosystem are unknown, but these changes accelerated after 1989 with the death of nine individuals. The social and genetic isolation of this group, the high levels contaminants in their blubber (Matkin et al 1998, Ylitalo et al. in prep), and the dramatic region-wide decline in harbor seals (Frost et al are factors that may be inhibiting their recovery.

Based the most recent genetic analysis and the association patterns we have observed over 15 years, it is doubtful that the AT1 group will interbreed with other known transient populations. The unique vocal repertoire and behavior of the AT1 transients supports this view (Saulitis et al in prep). Since there are few animals remaining (11 total) and there are possible genetic effects or behavioral constraints on inbreeding, the AT1s may be unable to recover unless a genetically similar population exists elsewhere and becomes available for interbreeding. This project is providing information that may lead to the listing of the AT1 group under the Endangered Species Act or as a depleted population under the Marine Mammal Protection Act.

The rate of encounters with killer whales in the Kenai Fjords region was again very high in 1999, due in great part to the tourboat sighting network; however, a bulk of the encounters were with AB (early season only), AK, AD05, and AK16 pods. Although the sighting rate was high, it was the smaller pods that were repeatedly seen, and we suspect the actual number of killer whales per day that used the area was much reduced from previous years. The larger well-known resident pods were seen infrequently, if at all. The AG pod and AF pods were not encountered. There was a relatively poor enhanced return

of coho salmon to the Kenai Fjords area in 1999 which may have been one reason that many of the larger resident pods were not seen or observed only briefly in the late summer/fall season. Reports from longline fishermen suggested that there may have been more killer whales offshore than usual at this time. Interestingly, a similar situation prevailed in British Columbia, where many of the B.C. northern resident pods were not encountered inshore, but were photographed 50 miles off Vancouver Island (G. Ellis, unpublished data).

Although we spent a limited time in Prince William Sound in 1999, the rate of encounter with killer whales was very low. Reports from Prince William Sound tourboat operators with whom we keep regular contact during the season indicated there were very few killer whales in the Sound during the summer and early fall 1999. Photographs and reports made opportunistically by those operators indicated that AK and AE pods and some AT1 transient group males were sporadically observed.

The continued analysis of genetic data generated over the past five years has further clarified the population structure of killer whales in the eastern North Pacific and described genetic variability within each population. These results underscore the need for management to address specific killer whale populations and take into account that unique populations may be sympatric. Although the AB pod is part of a much larger acoustic clan and population, the AT1 group is not, although their genetic variability suggests that they have relatively recently become a small remnant population.

Acoustic techniques for monitoring killer whales via remote hydrophones have been developed and improved over the past several years. We have switched to microwave transmission systems that are self sustaining using wind and solar generated power. The importance of proper location of the stations became apparent after severe difficulties with ambient noise experienced in winter 1997-98 at the Fox Island station. We were able to monitor the underwater acoustic environment in Resurrection Bay for a much greater period of time than previously during fall/winter 1999-2000. These results will be summarized in the FY 2000 report.

Although for many resident pods and for the AT1 transient group, dialects have been clearly defined, recordings of AG and AF pod dialects remain incomplete and field work in southeastern Alaska (the apparent center of their range) is planned in summer 2000 as part of a separately funded project. The concurrence of acoustic data and genetic data has been a valuable discovery. It gives us confidence that our acoustic separations determined for populations and within populations are useful for flield separation study of interrelationships.

As a result of the long-term investigations reported on here, as well studies n adjacent regions, it is clear that even the largest killer whale populations identified to date in the Eastern North Pacific number only in the hundreds of individuals. These populations should be considered at all times "vulnerable" because of their low numbers, low reproductive rates, and susceptiblity to anthropogenic as well as natural environmental perturbations. Because these small populations occupy a position atop the marine food chain and because of their potenial to accumulate toxic contaminants, killer whales should be considered a sentinel species that warrant careful long-term monitoring.

ACKNOWLEDGMENTS

Funding for work in 1989-1991 and in 1995-99 was provided by the *Exxon Valdez* Oil Spill Trustee Council via the National Marine Fisheries Service. Work in 1984 was supported by Hubbs SeaWorld Research Institute and field work in 1986 was supported by the Alaska Sea Grant Program and the National Marine Mammal Laboratory. Data for killer whale pods in PWS in 1985, 1987,1988,1992-94 was provided by the North Gulf

Oceanic Society. The Society was funded by private donations, the Alaska State Legislature and various State programs, the Alaska Sea Grant Program, Alaska Fund for the Future, the International Wildlife Coalition, and the American Licorice Company. The Vancouver Aquarium, Canada, provided use of their acoustic laboratory. We wish to thank Shayne MacLellan at the Pacific Biological Station for final identifications on fish scale samples. We also thank John Lyle, Kathy Turco and Olga von Ziegesar who provided field assistance in 1994 and 1995.

The following individuals helped make the genetic laboratory work possible: Martin Adamson, Amanda Brown, Fiona Buchanan, Jim Clayton, John Ford, Margaret Friesen, Kathy Heise, Sally Otto, Jamie Smith, and Rick Taylor. Special thanks are due Valentina Mendoza, who conducted the genetic sexing procedures.

Many people contributed recordings of killer whales in Prince William Sound and adjacent areas. Mike Brittain assisted with logistics and winter killer whale sightings. Special thanks goes to Kathy Heise for high quality recordings crucial to the success of this study. Photographs and assistance was also provided by NPS biologist Peter Armato and his co-workers at Kenai Fjords National Park

Without the assistance of the tourboat industry and the many skilled skippers based in Seward, Alaska the success we enjoyed in the field would not have been possible. We look forward to continuing our cooperative effort in the future.

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Appendix 1.

Reproductive isolation between sympatric and parapatric populations of killer whales in British Columbia and Alaska.

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Draft Manuscript for: Proceedings of the Royal Society, London B.

Introduction

The development of reproductive isolation is the critical step in any speciation event. Ernst Mayr (1942) argued that a period of allopatry was a strict requirement for speciation. This view has gradually changed, and the list of conditions and scenarios that could theoretically initiate or reinforce sympatric speciation is growing, as is the list of species divergences that likely occurred sympatrically. Sister species that are considered good candidates for sympatric speciation generally still have overlapping ranges, and are distinguished on the basis of morphological differences related to ecology and/or mating. It is inherently difficult to identify sympatric divergences at an early stage, before obviously-apparent morphological differences have evolved. Not surprisingly, the search for genetic subdivision in natural populations has nearly always been a search for barriers to migration.

In only two mammal species that we are aware of, humans and killer whales, is there evidence of sympatric reproductively-isolated, morphologically similar populations. In humans, ethnic groups have coexisted for many generations without fusing culturally or genetically. Gypsies, for example, have persisted in the midst of other European groups for centuries (Guglielmino 1996). Killer whales, the focus of this paper, were shown by Bigg (1982) to live in two sympatric but socially isolated populations along the coast of British Columbia and adjacent areas.

Numerous demographic and behavioural studies of killer whales followed Bigg's discovery (eg Ford 1989, Bigg et al. 1990 , Ford 1991, Baird and Dill 1996, Barrett-Lennard et al. 1996a, Matkin et al. 1997, Deecke et al. 1999). All these studies used photographs to identify individuals, and many focused on association patterns and group membership. It is now evident that the sympatric populations, referred to as *residents* and *transients*, feed on different prey, and differ in social structure and behaviour. Genetic studies by Stevens et al. (1989), Hoelzel (1991) and Hoelzel et al. (1998) found evidence of fixed genetic differences between the populations.

Residents prey on fish, principally salmonids. They occasionally harass marine mammals, but have not been seen to kill or eat them (Ford et al.

1998). Resident social organization is highly structured. Individuals travel throughout their lives in *matrilines*, comprising a matriarch and her complete lineage, both male and female. Matrilines usually contain 4-12 individuals from two to four generations, and often travel in association with other matrilines. It is believed that they associate most often with matrilines with which they share recent maternal ancestors (Bigg et al 1990). Groups of frequently-associating matrilines are known as *pods*. The largest unit of social structure is a set of associating pods that share a common range. Bigg (1982) and subsequent authors referred to this unit as a *community;* we refer to it here as a subpopulation. The pods in a resident subpopulation belong to one or more dialect groups, or *acoustic clans* (Ford 1991). Pods associate freely both within and between clans within their subpopulation, but do not associate with pods from other subpopulations (Bigg et al 1990).

In British Columbia, two subpopulations of residents have been studied for many years. The so-called southern resident (SR) subpopulation contains a single acoustic clan, and is most commonly sighted in the summer months in waters near the southern part of Vancouver Island. Its distribution in winter is unknown, although it has been sighted hundreds of miles away from its summering area, (G.M.E, unpublished data, N. Black, pers. comm.). The northern resident (NR) subpopulation contains three clans, and is usually sighted in coastal waters ranging from the central part of Vancouver to southern Alaska. A third resident subpopulation known as the southern Alaskan residents (SAR) is commonly sighted in the northern Gulf of Alaska has been studied since the early 1980's (Leatherwood et al. 1990). It contains at least two acoustic clans (Jurk et al. 1998). Details of the approximate size and distribution of each of the resident subpopulations are presented in Table 1 and Figure 1.

Transient killer whales prey on marine mammals, principally seals, porpoises, and sea lions (Ford et al. 1998). They occasionally kill seabirds, but have not been seen preying on fish (Ford et al. 1998). Transient killer whales have a more fluid social structure than residents. Although long-term associations between maternally-related individuals are common, individuals occasionally disperse (Ford and Ellis 1999). Transients travel in groups that rarely contain more than six individuals. They do not appear to have any functional equivalent of the resident pod or clan. As with residents, the transient population is divided into

discrete geographic subpopulations. Studies of transient dialects are at an early stage. However, the evidence to date suggests that each transient subpopulation has a single dialect, and that dialects vary between subpopulations (Ford 1984), (Saulitis 1993).

The best-known transient subpopulation, referred to as the west coast transients (WCT), ranges along the coast from Glacier Bay, Alaska, to central California (Goley and Straley 1994, Ford and Ellis 1999). In contrast, the small AT1 transient subpopulation has only been sighted in the waters in and near Prince William Sound, Alaska. A third, poorly-known subpopulation known as the Gulf of Alaska transients (GAT) inhabits the waters west of Glacier Bay. These whales enter Prince William Sound on occasion, but have not been seen to associate with the AT1's. The western extent of the GAT's range is unknown. It is rarely seen near the coast, except in exposed areas. The approximate sizes and distributions of the transient subpopulations are given in Table 1 and Figure 2.

In recent years, a third putative population of *offshore* killer whales has been identified (Ford et al. 1994; Table 1, Figure 1). Little is known about this assemblage, other than that it contains at least 200 individuals, is usually sighted 20 or more km from the mainland or Vancouver Island coastlines, ranges between California and the southern tip of Alaska, and typically travels in groups of 20 or more individuals. Hoelzel et al. (1998) found no differences in mitochondrial DNA sequences of offshores and southern residents.

This paper reports a comprehensive analysis of population segregation in killer whales off the west coasts of British Columbia and Alaska. Our objectives were to (1) re-examine the extent of reproductive isolation between residents and transients, (2) determine the extent of reproductive isolation between putative subdivisions of each population (3) ask whether the resident/transient separation occurred once or multiple times, (4) compare the levels of genetic diversity in residents and transients (5) determine the population status of the offshore group of killer whales, and (6) determine whether resident killer whales remain permanently within their natal groups, as suggested by field evidence. The study is based on the analysis of DNA from skin biopsies of photo-identified killer whales from British Columbian and Alaskan waters.

Table 1. Identity, estimated size and acoustic clan structure of known killer whale subpopulations in the north eastern Pacific.

Population	Sub-population	Abbrev.	Acoustic Clans	Appr. Size	Source
Resident	Northern	NR	A, G, R	209	Ford et al. 1994
	Southern	SR	J	82	K. Balcomb, pers. comm.
	Southern Alaskan	SAR	AB, AD	360+	Matkin et. al. (1999)
Transient	West Coast	WCT		220	Ford and Ellis (1999)
	Gulf of Alaska	GAT		60 +	Ford and Ellis (1999)
	AT1	AT1		11	Matkin et al. (1999)
Offshore		OFF		200+	Ford and Ellis (1999)

Sources are given for population size estimates. Acoustic clans of northern and southern residents described by (Ford 1991), and of southern Alaskan residents by Yurk et al. (in prep.).

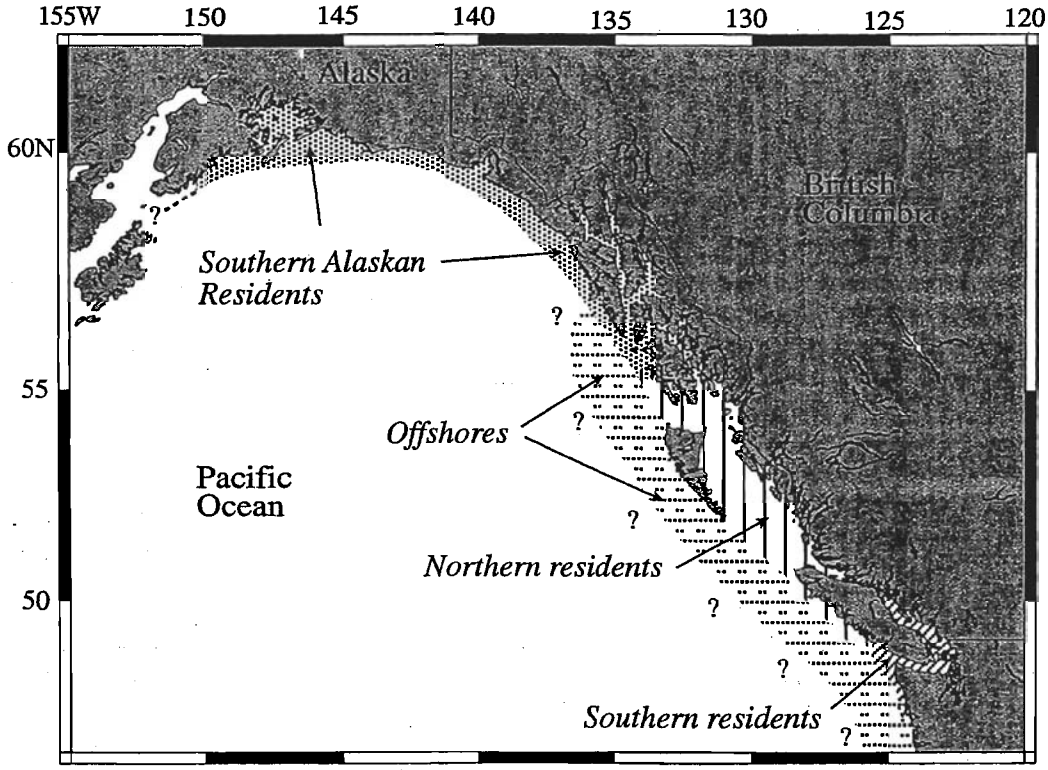

Figure 1. Approximate distributions of known offshore and resident killer whale populations in the north-eastern Pacific Ocean. These distributions are largely based on summer observations.

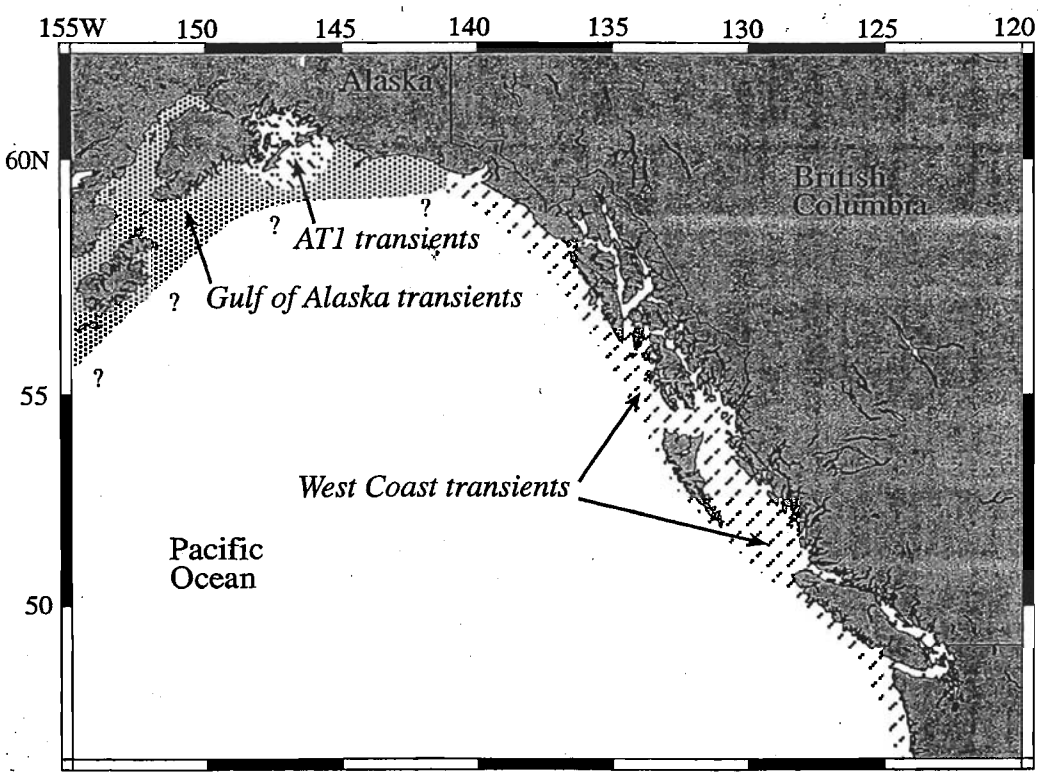

Figure 2. Approximate distributions of transient killer whales. The western extent of the distribution of Gulf of Alaska is conjectural—most sightings have been made between Kodiak Island and Prince William Sound.

Methods

Study areas, and biopsy procedures

We concentrated our biopsy sampling effort in two areas: in and around Prince William Sound, Alaska (59°30'-61°0'N, 146°15'-150°0'W), and from northern Vancouver Island to Caamaño Sound, British Columbia (50°45'-53°0'N, 127°0'-129°45'W). We also biopsied whales near Langara Island (54°14'N, 133°0'W) and in the western Strait of Georgia (49°15'N, 123°42'W). To locate whales, we visually searched areas where killer whale sightings were common by scanning with binoculars from a 6- to 8-m planing boat and from high points on shore. We also searched acoustically by listening for vocalizations with a directional hydrophone. Mariners often reported whale sightings to us by marine radio, helping us focus searches.

We approached killer whales that we encountered to a distance of approximately 25 m, matched their speed and course, and photographed as many individuals as possible for positive identification later (using identification catalogues by Bigg et al. 1987, Heise et al. 1992, Ford et al. 1994, Dahlheim et al 1997, Ford and Ellis 1999, Matkin et al. 1999b and G.M.E. unpublished data). We then chose an individual to biopsy that we could identify visually. First priority was given to members of matrilines that had not been previously sampled. We waited until the selected whale was travelling at a consistent speed and direction, so we could anticipate its surfacing and avoid confusing it with other individuals. We then approached slowly to 10-15 m, aimed for the back immediately behind the dorsal fin, and fired an untethered biopsy dart (Barrett-Lennard et al. 1996b). The darts were designed to bounce from the skin and float, and were retrieved from the water with a long-handled net. The biopsy sample was removed from the dart tip and the skin portion was stored in a sterile solution containing dimethlysulphoxide and NaCl (Amos, W. and Hoelzel 1991) at 4°C. The blubber portion was preserved separately for contaminant analysis (results in (Ross 2000).

To extract DNA, we homogenized 30 mg of skin in a glass tissue grinder, digested it with proteinase K for 24 h at 54°, purified the DNA with phenol and chloroform and precipitated it with ethanol using standard procedures (Sambrook et al. 1989). Care was taken to prevent cross-contamination by using sterile disposable labware where possible, flame- or acid-sterilizing non-disposable items, and using aerosol-filtered

pipettor tips during all procedures. The purified DNA was dissolved in TE buffer, and the approximate concentration of DNA in this stock solution determined with UV spectrophotometry (Sambrook et al. 1989). The DNA stock was stored at -80°C. We made a working solution of each sample by diluting a portion of its stock solution in water to a DNA concentration of 50 ng/ul. This working solution was stored at -20°C, and was replenished as required from the stock.

Mitochondrial DNA analysis

We selected one individual for mtDNA sequencing from each maternal lineage (based on Ford et al. 1994 and Matkin et al. 1999a) that we had biopsied. To conduct the sequencing, we (a) used the polymerase chain reaction (PCR) to amplify the entire D-loop region using custom-designed primers annealing to the tRNA-Thr and 12s-rRNA regions (primer sequences based on Commerson's dolphin (Southern et al. 1988), and fin whale sequences (Arnason et al. 1991), (b) purified the samples with QIAQuick® spin columns and protocols supplied by Qiagen, Ltd., (c) ran sequencing reactions using Fs-Taq® system reagents and protocols supplied by Applied Biosystems, Ltd., and (d) resolved the sequences using an Applied Biosystems 377 automated DNA sequencer. Because the sequence was too long (950 bases) to be entirely resolved in one direction, we ran sequencing reactions from each end of the amplified fragment.

We visually checked the graphs of nucleotide order and band strength from the automated sequencer and corrected the computer-generated sequences accordingly. We also used the approximately 400-base overlap in the sequences of opposite directions to check for errors. As a final accuracy check, we confirmed differences between sequences from different individuals by comparing their sequence output graphs. We then aligned unique sequences using the program CLUSTRAL-W (Thompson et al. 1994).

Microsatellite Analysis

We tested 27 primer sets developed for microsatellite analysis in cetacean species (Amos, B. et al. 1993, Buchanan et al. 1996, Richard et al. 1996, Valsecchi and Amos 1996, Hoelzel et al. 1998) for their ability to amplify microsatellite loci in killer whales. In this testing process, we ran low-stringency PCR reactions (Innis and Gelfand 1990), electrophorosed the PCR products on 1.2% agarose gels, stained them with ethidium bromide,

and photographed them under UV light. When a given primer set produced an amplification product that was similar in size to that described in its original study, we used an empirical optimization procedure (Innis and Gelfand 1990) to improve the selectivity and yield of the reaction. We then used the following procedures to visualize the amplified DNA with greater precision: 1) one of the primers was end-labelled with [γ-^{33}P] by incubating 50 pmol primer, 10 units polynucleotide kinase, and 10 uCi [γ-^{33}P]ATP in PNK buffer for 35 min at 37°, 2) PCR was performed under the optimized conditions using 1 pmol of labelled primer, 2.5 pmol of the same primer unlabelled, and 6 pmol of the reverse primer, and 3) the PCR products were resolved on a 0.4 mm X 30 cm X 40 cm denaturing gel containing 5% polyacrylamide, dried on filter paper, and exposed to autoradiograph film for 12 to 96 hours. We identified microsatellite DNA on the developed film by the presence of characteristic shadow bands (Hauge and Litt 1993), and determined alleles sizes by comparing the bands to reference DNA sequences run on every gel.

We initially tested each pair of primers on DNA from 40 killer whales that we believed to be unrelated, including resident and transient individuals from both British Columbia and Prince William Sound. Those primer pairs that produced clear microsatellite bands and that revealed at least three different alleles in the test group were used to type all biopsied killer whales; we conducted no further analyses with remaining primers. During the routine typing at each of the selected microsatellite loci, samples that failed to amplify or that produced ambiguous bands on the gel were amplified a second and if necessary a third time. We scored the alleles manually by comparison to the reference sequence. As a check, we re-scored each film several days later, and then compared the two sets of scores. As an additional check of the consistency of scores, we re-amplified a minimum of 5% of the samples at each locus, and scored them two more times.

Data Analysis

Mitochondrial DNA

We inferred historical relationships among the haplotypes using a branch-and-bound search algorithm to find optimal trees based on a maximum-likelihood criterion (Swofford et al. 1996); calculations performed using PAUP* version 4.0b2a, (Swofford 1998). The maximum likelihood analysis used nucleotide base frequencies and transition/transversion ratios based

on the D-loop sequences. We repeated the analysis on 100 bootstrapped versions of the data, and constructed a tree based on the consensus.

Microsatellite DNA

We grouped the data based on population subdivisions suggested by observational data (Bigg et al. 1990, Ford et al. 1994, Barrett-Lennard et al. 1995), the mitochondrial analysis described above, or both. Using the microsatellite genotypes from the group with the greatest sample size, we tested each locus for evidence of heterozygote deficiency using Guo and Thompson's (1992) (Guo and Thompson 1992) Markov chain method as implemented in GENEPOP (Raymond and Rousset 1995). Gene diversities were calculated for each locus in each subpopulation using Nei's (1987) unbiased formula $H_e = \frac{2n}{2n-1}(1-\sum_{i=1}^{k} p_i^2)$. To compare gene diversities between residents and transients, we used a nested two-way ANOVA, with population and locus as factors and with subpopulations nested within populations. We also calculated Weir and Cockerham's (1984) estimators of Wright's F-statistics for the subpopulations using the program FSTAT 2.8 (Goudet 1995), and performed 1000 bootstraps across loci to determine 95% confidence intervals for the estimates.

We calculated Nei's standard genetic distance D_s (Nei 1972) between all putative subpopulations using the program MICROSAT (Minch et al. 1995). D_s does not assume any particular mechanism of mutation, unlike recently-developed measures which assume that mutation occurs in a stepwise fashion (eg $\delta\mu 2$, (Goldstein et al. 1995). Stepwise mutation-based measures are expected to be linear with respect to time at longer time scales, whereas D_s is considered a more appropriate measure when divergences have taken place recently (Goldstein et al. 1995, Paetkau et al. 1997).

The genetic distance matrix was used to construct a UPGMA (unweighted pair group method with arithmetic averaging) phylogram, using the program PAUP* (Swofford 1998). This tree indicates the relative similarity of each subpopulation but, since UPGMA is effectively a cluster analysis, it is not an explicit reconstruction of population phylogeny. We used this approach since the history of the subpopulation divergences is likely reticulate, due to occasional inter-group matings (see (Smouse 1998), not strictly radiate as assumed by phylogeny-reconstruction algorithms.

Results

Biopsy Samples

We acquired skin samples by biopsy dart from164 identified killer whales from British Columbia and 97 from Prince William Sound, Alaska. In addition, we obtained skin, muscle or bone samples from the stranded carcasses of seven identified killer whales from British Columbia and immediately adjacent waters, and one from Prince William Sound. For comparison purposes, we also obtained five tissue samples from killer whales from the northern and southern Atlantic ocean. The origin of each whale sampled is listed in Table 2.

Mitochondrial DNA

A total of 134 killer whales were sequenced, one from each matriline. that we had biopsied. We identified 11 variable sites in these sequences, comprising one single base-pair insertion/deletion, nine transitions, and one transversion. The Atlantic killer whales added two additional variable sites, both transitions. These 13 variable sites defined ten haplotypes.

The three transient subpopulations, the offshores, and the two British Columbian resident subpopulations were each monomorphic for different haplotypes. In contrast, the Prince William Sound resident subpopulation had two haplotypes, one matching the southern B.C. residents, and the other the northern B.C. residents. Pod members always shared a single haplotype, but pods with different haplotypes were frequently seen in close association. A maximum likelihood tree based on the D-loop sequence data is presented in Figure 3.

Table 2. Identities of sampled whales.

Population	Putative Subpopulation[1]	Acoustic Clan	Pod
resident (215)	Southern Residents (8)	J	J1 (7)
			L1 (1)
	Northern Residents (126)	A (75)	A1 (17)
			A4 (10)
			A5 (15)
			B1 (8)
			C1 (8)
			D1 (4)
			H1 (5)
			I1 (1)
			I2 (3)
			I18 (4)
		G (34)	G1 (7)
			G12 (7)
			I11 (14)
			I31 (6)
		R (17)	R1 (15)
			W1 (2)
	Southern Alaskan Residents (82)	AB (44)	AB (14)
			AG3 (3)
			AI (6)
			AJ (12)
			AN (8)
			AX (1)
		AD (38)	AD5 (4)
			AD16(4)
			AE (15)
			AK (12)
			AS (3)
offshore (7)	[British Columbia and SE Alaska]		
transient (46)	West coast transients (30)		
	Gulf of Alaska transients (8)		
	AT1 transients (8)		
Atlantic (5)	[West coast of France] (2)		
	[Iceland] (2)		
	[Southern Brazil] (1)		

Numbers of samples in each category in round brackets. Whale identifications and pod designations based on (Heise et al. 1992, Ford et al. 1994, Ford and Ellis 1999, Matkin et al. 1999b). Acoustic clan designations for British Columbia residents from Ford et al. 1994. Offshore killer whales identified by GME. Tissue samples from identified carcasses were provided by D. Bain (two offshore samples) and D. Nagorsen (L1 pod sample). Icelandic, French and Brazilian samples provided by C. Wright, A. Collet, and E. Secchi respectively. [1]Where putative subpopulation unknown, sampling location is given in square brackets.

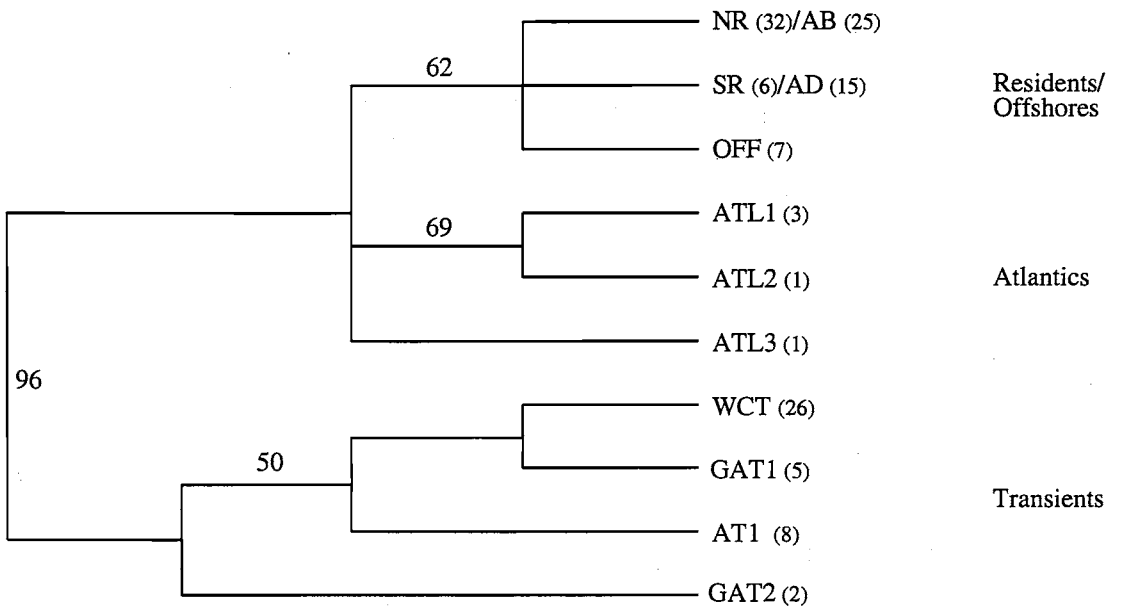

Figure 3. Consensus maximum likelihood tree based on mitochondrial D-loop sequences for seven Pacific and 3 Atlantic killer whale haplotypes.

Numbers on branches indicate percentage bootstrap support. The number of whales sequenced in each category is shown in brackets. AB and AD refer to two clans of southern Alaskan residents (see Table 2). Abbreviations as in Table 1, except ATL1 = 2 killer whales captured near Iceland and one that stranded on the west coast of France, ATL2 = a stranded killer whale from the Brazilian coast, ATL3 = a stranded killer from the west coast of France, GAT1= 6 of the 8 sequenced GAT whales, GAT2 = the remaining GAT whales sequenced, AT1 = AT1 transient group of Prince William Sound. In calculating the tree, the single insertion/deletion was accorded the same probability as a T/C transition, however its exclusion from the data did not affect the tree topology.

Microsatellite DNA

We tested twenty seven sets of PCR primers (developed by Schlötterer et al. 1991, Buchanan et al. 1996, Valsecchi and Amos 1996, and Hoelzel et al. 1998). Five failed to amplify microsatellite DNA, and four amplified but were monomorphic. Seven amplified fewer than three alleles in the test data set or produced ambiguous bands, leaving 11 readily-scoreable polymorphic loci (Table 3). We amplified all 285 DNA samples in the dataset (Table 2) at these 11 loci. The DNA from dart biopsies was relatively unsheared, and the proportion of missing scores across all loci and all samples was 0.004. The DNA from carcasses was generally more degraded, and the proportion of missing scores in these samples was 0.174. None of the 11 loci was sex-linked, as heterozygous individuals of both sexes were scored.

The number of alleles per microsatellite locus in the resident, transient and offshore populations ranged between 3 and 20, with a mean of 7.8. Tests for heterozygote deficiency in the largest putative sub-population sampled, the northern residents, were negative for all 11 loci, with p values ranging between 0.27 and 0.91. The size and distributions of the alleles at each locus are presented in Appendix 1, and the gene diversities in Table 3. Gene diversities were significantly greater in transients than residents ($F_{1,50} = 12.66$, $p = 0.0008$).

Estimates of Wright's F-statistics for the three resident subpopulations and the three transient subpopulations are presented in Table 4. Pairwise Fst estimates for each of the subpopulations are in Table 5. Figure 4 is a phylogram of the seven subpopulations based on genetic distances.

Table 3. Gene diversities and total number of alleles at 11 microsatellite loci in seven subpopulations of killer whales from Alaska and British Columbia.

Locus	1	2	3	4	5	6	7	8	9	10	11	Mean
SR	0.473	0.384	0.648	0.000	0.627	0.142	0.560	0.362	0.142	0.473	0.560	0.398
NR	0.718	0.550	0.421	0.277	0.399	0.229	0.499	0.432	0.443	0.510	0.612	0.463
SAR	0.545	0.692	0.337	0.234	0.533	0.486	0.494	0.371	0.501	0.577	0.631	0.491
OFF	0.704	0.670	0.264	0.142	0.473	0.264	0.528	0.660	0.264	0.637	0.660	0.479
WCT	0.792	0.733	0.419	0.437	0.815	0.577	0.736	0.711	0.664	0.683	0.742	0.664
GAT	0.879	0.705	0.663	0.358	0.810	0.489	0.758	0.800	0.753	0.780	0.716	0.701
AT1	0.686	0.543	0.699	0.568	0.000	0.503	0.503	0.000	0.523	0.607	0.000	0.421
Alleles[1]	20	9	6	3	8	4	6	7	6	8	9	7.8

[1]Total number of alleles in all seven subpopulations.

Table 4. Weir and Cockerham (1984) estimators of *F*-statistics combined over 11 microsatellite loci for killer whale sub-populations from Prince William Sound, Alaska and British Columbia†.

	Fis	Fst	Fit
all subpopulations	-0.014	0.205	0.194
[6]	(-0.049 - 0.022)	(0.140 – 0.269)	(0.114 – 0.276)
resident	-0.019	0.088	0.070
subpopulations [3]	(-0.056 - 0.020)	(0.032 - 0.146)	(0.114 – 0.276)
transient	0.004	0.167	0.170
subpopulations [3]	(-0.096 – 0.086)	(0.088 – 0.241)	(0.073 – 0.236)

† Subpopulations as listed in Table 1. Round brackets indicate ninety-five percent confidence intervals for each estimator; square brackets the numbers of subpopulations in each analysis.

Table 5. Weir and Cockerham (1984) estimators of Fst, for each pair of sampled subpopulations of killer whale from Prince William Sound and British Columbia. The probabilities that the statistics were not greater than zero, based on permutation tests, were less than 0.001 in every case.

NR	0.144					
SAR	0.187	0.076				
OFF	0.321	0.278	0.305			
WCT	0.229	0. 278	0.259	0.153		
GAT	0.226	0.251	0.234	0.182	0.065	
AT1	0.429	0.430	0.399	0.422	0.224	0.290
	SR	NR	SAR	OFF	WCT	GAT

Abbreviations as in Table 1. For testing for Fst differences from zero, multi-locus genotypes were permuted among subpopulations 10,000 times.

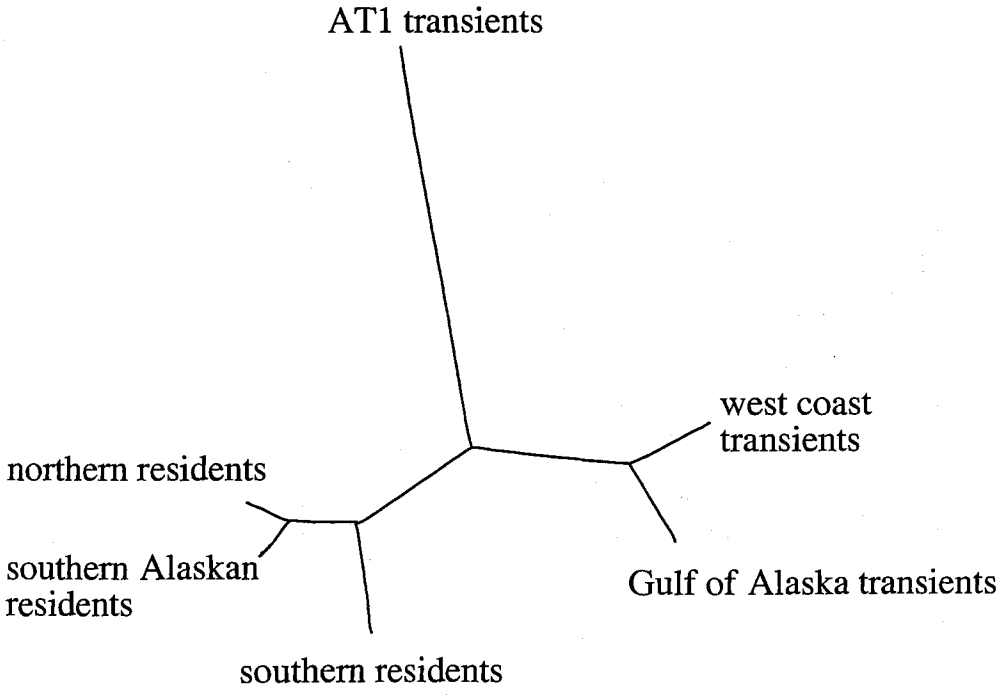

Figure 4 . Unrooted UPGMA phylogram for Alaskan and British Columbian killer whales based on 11 microsatellite loci, using Nei's standard genetic distances.

Discussion

The present study builds on the findings of earlier genetic analyses of killer whales in the northeastern Pacific, (Stevens et al. 1989, Hoelzel 1991, Hoelzel et al. 1998), but differs from them in the following ways. (1) The number of samples that we collected and analysed was several times greater than in any earlier study. (2) We attempted to positively identify all individuals prior to biopsying them, and excluded individuals of uncertain identity from our data sets. This approach allowed us to test the extent of genetic differentiation among four putative subpopulations that had not been previously examined. (3) We attempted to biopsy at least one whale from each known maternal lineage of killer whales in the study area, so that each population was as broadly represented as possible. Previous studies used multiple samples from a small set of maternal lineages. (4) The length of mitochondrial DNA sequenced and the number of microsatellite loci typed were substantially greater than in earlier studies.

Our findings have six significant implications. (1) The resident and transient killer whale populations are independent, reproductively isolated lineages. (2) Both populations are subdivided into regional subpopulations between which migration is restricted. (3) Fish-eating and mammal-eating traditions diverged once in the northeastern Pacific. (4) Gene diversity is significantly lower in residents than in transients. (5) The recently-identified offshore group of killer whales is genetically differentiated from both residents and transients. (6) Our findings support field studies indicating that resident individuals remain within their natal groups for life. We expand on each of these points below.

Resident and transient killer whale lineages are reproductively isolated.

The analysis of mitochondrial sequences identified two well-defined clades, one containing all of the transient subpopulations, and one containing all of the residents. Individuals classified *a priori* as resident and transient had no haplotypes in common. Since the classifications came from observational studies alone, and in view of our substantial sample sizes, we are confident that female migration between the two forms is at most extraordinarily rare. Comparisons of mitochondrial and nuclear microsatellite DNA—inherited from mothers only and from both parents, respectively—are often used to test for sex-biased dispersal. In

this case, however, the general patterns are similar: the microsatellite phylogram (Figure 4) preserves the separation of residents and transients, pairwise Fst values (Table 5) are much higher between resident and transient subpopulations than between subpopulations of a common population, and several loci have population-specific alleles (Appendix 1). These results suggest that male dispersal may be more common than female dispersal, but it must still be rare.

The resident and transient populations differ by 7-9 substitutions in the D-loop region, an absolute divergence of 0.8-1.0%. It is tempting to date the time of the divergence using a molecular clock approach that assumes a constant substitution rate over time. Unfortunately, such methods are notoriously imprecise (Hillis et al. 1996, Messenger and McGuire 1998). This is especially true for divergences within a species, where haplotypes could easily diverge and persist for long periods within a single population. We thus draw no conclusions about when the segregation may have arisen.

The more interesting question of whether the divergence of mammal-hunting and fish-hunting specialists occurred long enough ago for adaptive evolution to have occurred could be answered directly. For example, tooth size or jaw robustness likely have different optima in mammal and fish hunters. Evidence that these traits are variable and heritable, yet not significantly different between the populations, would favour a recent origin of the feeding specialist divergence. In any event, there is little reason to think that residents and transients are reproductively incompatible (that is, separated by post-mating isolating mechanisms). Both have been successfully crossed with Icelandic whales in captivity (whale identities from Hoyt, 1984; mating records from Duffield et al., 1995), and it is likely that they would produce viable offspring if crossed together.

The resident and transient populations are divided into genetically differentiated regional subpopulations.

Stevens et al. (1989) and Hoelzel (1991) described mitochondrial DNA differences between southern and northern residents. This study is the first attempt to specifically test for genetic differentiation between putative subpopulations of transients, and to test whether the southern

Alaskan residents are reproductively isolated from the other resident groups.

The finding of fixed mitochondrial differences between the northern and southern residents effectively rules out female-mediated gene flow between the two subpopulations (Figure 3). The microsatellite analysis (Table 4, Figure 4) showed that they are also strongly differentiated at nuclear loci, indicating that male-mediated gene flow is also small at best. Although the two subpopulations are usually spatially separated in the summer, little is known about their travel patterns in winter. Two of the SR pods have been sighted several times in the spring travelling towards their summer feeding grounds through Johnstone Strait, a core area for the northern residents. Thus, it is very likely that members of the two populations come into acoustic and perhaps visual contact at least occasionally, suggesting that their reproductive isolation results from social factors.

The SAR residents have two mitochondrial DNA haplotypes, one matching the SR haplotype and one the NR haplotype, suggesting that it shares recent maternal ancestors with both groups. The SAR microsatellite genotypes indicate relatively weak segregation from the NR's, and much stronger segregation from the SR's, as reflected in the Fst values in table 5 and the UPGMA analysis in Figure 4. These patterns suggest that limited gene flow between the NR and SAR groups may occur, but that little if any occurs between the SR and SAR groups. Two pods of SAR whales are commonly seen in southern Alaska, and have been seen on at least one occasion associating with two northern resident pods (Dahlheim et al. 1997), indicating that the social isolation of the NR and SAR subpopulations is not complete.

The situation with transients is somewhat similar. The WCT and AT1 groups each had a single unique haplotype; two haplotypes were found in the GAT group. The three groups have mitochondrial sequences that are clearly more similar to each other than they are to any other group. The sequences are evidence that female dispersal between subpopulations is rare at best. We note, however, that our sample of GAT's is small, and it is possible that AT1 or BCT haplotypes will be discovered with more sampling effort. At microsatellite loci, Fst estimators suggest weakly-restricted to gene flow between the BCT and GAT groups, and much stronger separation between both groups and the AT1's. The AT1 data

may be biased by close levels of relatedness between sampled individuals, however, the finding is consistent with the fact that this well-studied group has never been seen associating with GAT whales.

Fish-eating and mammal-eating killer whale assemblages diverged once in the northeastern Pacific.

In this study, biopsy sampling was conducted in two regions, British Columbia and Prince William Sound, Alaska. The terms resident and transient were first applied to killer whales in British Columbia. The same terms were later used to classify killer whales in Prince William Sound because of obvious behavioural parallels (Leatherwood et al. 1990). No individual killer whales have been sighted in both areas, and it was not known whether the divergence into mammal-hunting and fish-hunting specialist groups had occurred once or multiple times. Both the nuclear and the mitochondrial DNA analyses presented here are consistent with reciprocal monophyly, implying a single divergence. The initial divergence could have occurred sympatrically or allopatrically, however, the reproductive isolation described here suggests that the divergence is now widening in sympatry.

The best evidence of feeding specializations in killer whales from regions other than the eastern Pacific comes from (Berzin and Vladimirov 1983) who described morphologically-distinct fish-hunting and mammal-hunting killer whales in Antarctic waters. At Crozet Archipelago (46°S., 52°E.) in the southern Indian ocean, photo-identified killer whales that have been observed feeding on elephant seals have also been seen feeding on fish (Guinet 1990, 1992). Thirteen killer whales biopsied in the Crozet Archipelago had very similar mitochondrial D-loop sequences to the resident clade (LBL and C. Guinet, unpublished data), as did a whale that stranded in southern Brazil and two that stranded on the coast of France (LBL unpublished data). The Icelandic whales described in this chapter were also similar to the residents. In contrast, no killer whales with similar D-loop sequence to transients have been found outside the north-eastern coastal Pacific.

Mitochondrial and microsatellite DNA diversity is higher in transients than transients.

Whitehead (Whitehead 1998) noted that low mtDNA diversity typifies

cetaceans that live in social groups with little or no female dispersal, and proposed that mtDNA hitchhikes on cultural innovations that are strictly confined to social groups and which greatly increase the fitness of their members. The hypothesis predicts that transients should have higher mtDNA diversity than residents, since female dispersal occurs in the former but not in the latter. Amos (Amos 1999) offered a alternative explanation with less restrictive assumptions: the effective population size of mitochondria is a function of the number of matrilines, not of the census size, in strictly matrilineal species. We found higher levels of mtDNA variation in transients than residents (4 haplotypes in 3 putative subpopulations and 2 haplotypes in 3 subpopulations, respectively). This difference is consistent with the differences in social structure between the two populations, since residents remain within their matrilines for life (Bigg et al. 1990) whereas some transients of both sexes disperse (Ford and Ellis 1999).

Microsatellite DNA diversity was also significantly higher in transients than residents. This difference may indicate that the mean subpopulation size of transients is larger than that of residents. However, more residents than transients have been photo-identified and catalogued, and even though transients are more difficult to census than residents((Ford and Ellis 1999), it seems unlikely that large numbers of coastal transients have not been identified. Alternatively, transient subpopulations may be less closed to gene flow than residents, and diversity may be augmented by occasional matings between transients and either offshores or unknown subpopulations of killer whales. Finally, the patterns could result from historical contingencies—bottlenecks or founder effects in residents. However, we have no evidence of these events, and favour the first two explanations. (A third category of explanation, that residents are more inbred than transients, is ruled out in Barrett-Lennard et. al., in prep).

Offshores are genetically differentiated from all known resident and transient subpopulations.

Each of the offshore killer whales that we sampled had the same mitochondrial haplotype, which was closely related to the resident haplotypes. Clearly, residents and offshores share more recent maternal ancestors with each other than either does with transients. We found the opposite pattern at microsatellite loci. Here, offshores and transients

were most similar (Figure 4, Table 5). This pattern is consistent with three very different scenarios of historical and contemporary gene flow. First, offshores may have diverged from the same stock as residents, but have occasionally mated with non-offshore males, usually transients. Second, offshores may have diverged from the same stock as transients, and experienced mitochondrial DNA introgression after one or more resident females emigrated into the group. Third, offshores may have arisen from resident/transient hybrids. In view of the extremely strong propensity of contemporary resident females to stay in their matrilines for life (Bigg et al. 1990, this study), and to mate not only within their population but within their subpopulation (Barrett-Lennard et al., in prep.), we suspect that the first scenario is most likely. We note, however, that our sample size of offshores was small, and our picture of the relative relatedness of residents, transients and offshores may change as more samples are acquired.

Resident females remain within their natal groups.

One of the most striking finding to emerge from nearly thirty years of field studies of resident killer whales is the absence of dispersal of members of either sex from their natal matrilines. Here we asked whether the lack of dispersal over this period is typical of the recent history of the population, or whether dispersal rates have fluctuated. The SAR resident group consists of pods belonging to acoustic clans, each of which is fixed for a different mtDNA haplotype. Pods associate independently of clan membership, so individuals are in frequent social contact with members of other clans. There is little nuclear DNA differentiation of the two clans (Figure. 4), and strong evidence that inter-clan matings are common (Barrett-Lennard et al., in prep). If females disperse even rarely between pods, the observed relationship between clan membership and mitochondrial haplotype would quickly break down. We conclude therefore that dispersal by female residents has been rare for many generations. Mitochondrial comparisons cannot detect historical trends in male dispersal, but can identify males that have dispersed between populations with fixed haplotypic differences. We found no male dispersers among the SAR residents, and conclude therefore that male dispersal is rare at most in the present generation.

Acknowledgements

Many people made important contributions to this study. Kathy Heise and Eva Saulitis were of essential assistance throughout. Tom Smith made the British Columbian portion of the project possible, and was a great source of encouragement and assistance. We also acknowledge the invaluable support and help of Jamie Smith, Andrew Trites, Martin Adamson, Rick Taylor, Claire Thompson, Theresa Burg and Claire Thompson. Funding was provided by the Exxon Valdez Trustee Council, the Vancouver Aquarium Marine Science Centre's Killer Whale Adoption Program, and the Canadian Department of Fisheries and Oceans. L.B.L. was supported by fellowships from the Natural Sciences and Engineering Research Council of Canada and the University of British Columbia.

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Appendix 1

Distribution of alleles at 11 microsatellite loci for 7 subpopulations of killer whales.

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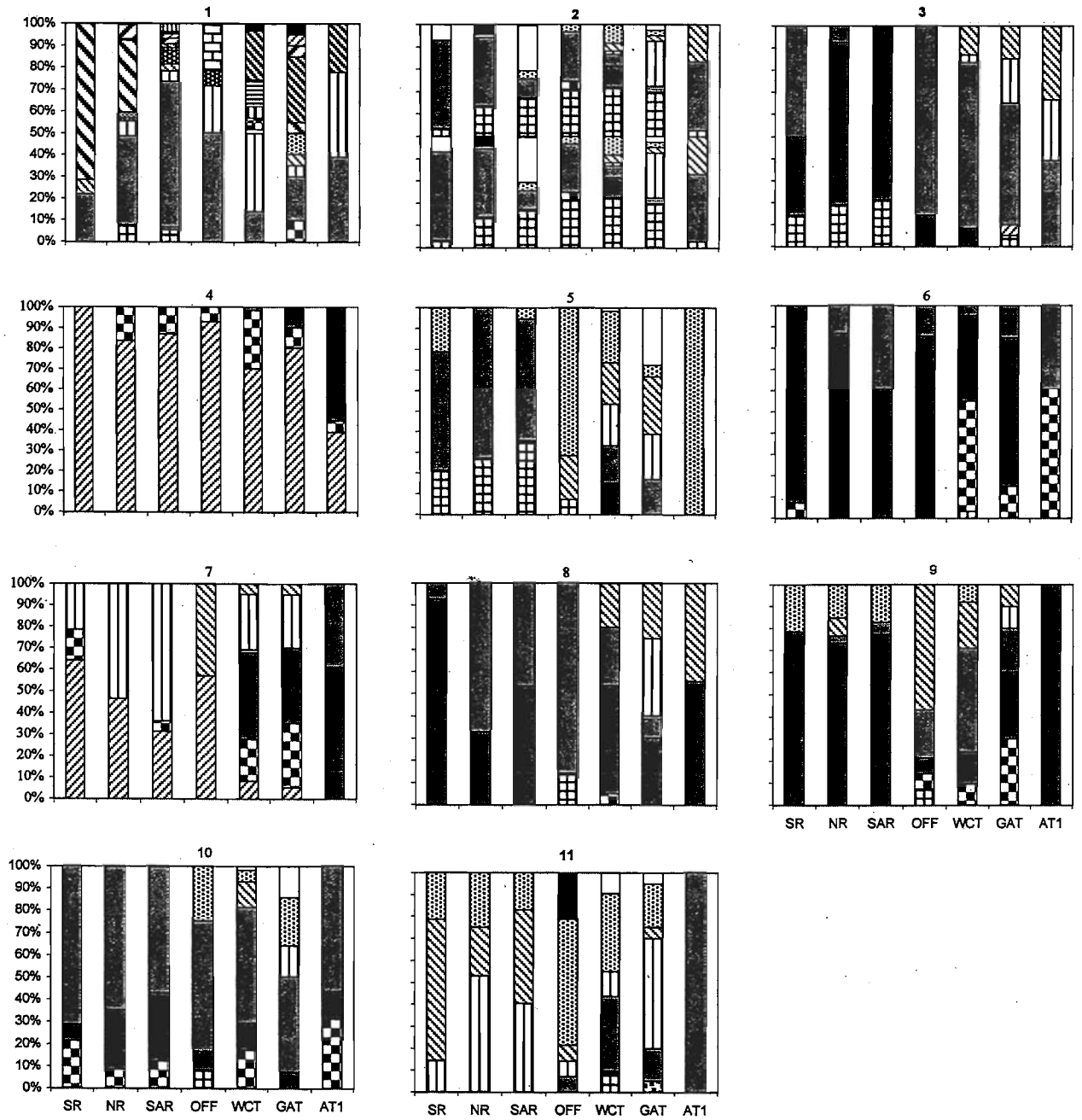

Appendix 2

Parallel cultural and genetic lineages in resident type killer whales off the coast of Southern Alaska

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Abstract

We present evidence that at least two acoustically and genetically distinct clans (vocally related pods) of resident killer whales inhabit Prince William Sound, Alaska. We compared the sound contours of 9000 randomly-chosen calls from 448 recording sessions of seven photo identified killer whale pods. The pods fell into two acoustically distinct clans, with no evidence of sharing of call types between them. One clan referred to as AB-clan, included AB, AI, AJ and AN pods. The second clan, AE-clan, included AD, AE and AK pods. We identified a mean number of 12 distinct call types for each pod, based predominantly on pulsed tone components. Call types and their variants were shared among member pods of the same clan. A dendrogram based on a quantitative index of acoustical similarity shows that within AB-clan, AB, AI and AN pods are vocally more similar to each other than either is to AJ pod. Within AD-clan, AD, AE, and AK pods are equally similar. Using DNA from biopsy samples, we sequenced the entire mitochondrial region control region of 21 AB-clan and 12 AE-clan individuals, including members of each pod. Each clan was monomorphic for a single haplotype and the two clans differed by one transition. It thus appears that the acoustic differences between the clans, which we presume to be cultural, are distinct clans (vocally related pods) of resident killer whales inhabit Prince William Sound, Alaska.

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Table 1: Pod encounters with analyzed recordings of six pods and number of biopsies samples collected from these pods in each year. Actual recording duration differed among encounters, so did vocal activity.

Table 2: List of all identified call types and variants (sub-types) in alphanumerical order. Call types that are produced by an individual pod are indicated by an X in the appropriate column. Pods that share call types are grouped together.

Table 3: Acoustic similarity between pod repertoires based on an index that represents similarity as a value between 0 and 1, where 1 means the repertoire of two pods are identical and 0 means the two pods do not share any call.

Introduction

The transmission of information between individuals of the same or different generations through social learning is cultural transmission (Dawkins 1976; Bonner 1980; Mundinger 1980; Cavalli-Sforza and Feldman 1981; Boyd and Richerson 1985, Lynch 1996, p.181). Cultural traditions arise from cultural transmissions across generations. Mundinger (1980, 1982) argued that because cultural transmission is phylogenetically widespread it must have evolved naturally. He proposed a general theory of cultural evolution that linked the complex social cultures of humans with simpler cultures found in non-human societies. In his theory, Mundinger (1980) defined cultural traditions as learned complexes persisting in the behaviour repertoire of individuals of a population for several generations. Furthermore, similar traditions can be traced back to a common ancestral behaviour. Although cultural transmission of learned traits had been discussed earlier (Cavalli-Sforza and Feldman 1973) it had been largely done in the context of human behavior and certain non-human primate behaviors (Kawamura 1959; Green 1975).

Cultural transmission has now been implicated in a number of observations of recurring stable behavious. The best-described examples are still found among non-human primates (Whiten et al. 1999; Masayuki et al. 1998; McGrew 1998; Alp 1997; Huffman 1997; Boesch et al. 1994; Eishi et al. 1994; Wrangham et al. 1994; Goodall 1986; Nishida 1986). Chimpanzees in Gombe and Mahale National Parks in Eastern Africa learn how to fish for termites and other insects, from their mothers or other adults and pass this knowledge on to their offspring (McGrew 1982; Nishida et al. 1983; Goodall 1986). On the other side of the continent, chimpanzees in Tai National Forest in West Africa have learned the value of using stones to open nuts (Boesch et al. 1994). Carrying an effective rock-tool spread quickly through the population, and appeared to have been transmitted across generations. Perhaps the most popular example of

cultural transmission outside the hominid family is that of the sweet potato washing Japanese macaques (Kawamura 1959). The behaviour has been invented by a female macaque named Ino in 1958, and spread over a period of two years first to related members of the troop and later to non-relatives. Ino also invented other behaviours, such as separating seeds from dirt by throwing it into the water and skimming the floating seeds off the surface (Nishida 1986).

Cultural traditions also occur in other mammals and in birds (Bonner 1980), and often involve learned vocal traits. These traits include song types, phrases or notes produced by many songbirds (Baptista 1975; Slater and Ince 1979; Mundinger 1982; Baker and Jenkins 1987; Trainer 1989; Slater and Williams 1992; Payne et al. 1985, 1996, p.198) as well as song types and themes of humpback whale songs (Payne et al. 1983). Discrete calls produced by killer whales (Ford 1991), and discrete temporal patterns in click vocalizations of sperm whales (Weilgart and Whitehead 1997) are transmitted through teaching and learning and belong also into this category. Most of these cultural traditions are commonly called dialects (Marler and Tamura 1962; Baker 1975; Baptista 1975; Mundinger 1980; Connor 1982; Baker and Cunningham 1985; Ford 1991).

Historically, both regional acoustic variations between populations, and acoustic variations among neighbouring groups that potentially mix and interbreed have been called vocal dialects. In the first scenario they are passive results of the geographic isolation of populations (Mundinger 1980, 1982; Conner 1982; Lynch 1996, p.181), and do not appear to function socially. In the second scenario, dialects could play a role in the biological evolution of densely packed animal societies (Marler and Tamura 1962; Baptista 1975; Conner 1982; Krebs and Kroodsma 1980; Baker and Cunningham 1985; Ford 1991; Weilgart and Whitehead 1997). Dialects then appear to reinforce assortative

mating in order to avoid inbreeding or outbreeding depression (Treisman 1978; Krebs and Kroodsma 1980; Mundinger 1982; Baker and Cunningham 1985). However, other studies suggest that most bird dialects are population epiphenomena because they result only from cultural mutation and drift, and are therefore selectively neutral with regard to biological evolution (Slater and Ince 1979; Payne 1985; Williams and Slater 1992; Lynch 1996, p.181: Payne 1996, p.198). These studies argue that dialects are not adaptive because individuals do not directly benefit from adopting a particular one.

Dialects of the second type or socially functional dialects are generally rare communication features among animals. There is little evidence for such dialects in the communication systems of songbirds (Baker and Cunningham 1985), and aside from pictail macaques (Gouzoules and Gouzoules 1990), spear-nosed bats (Wenrick Boughman 1997), and humans, the only other mammals in which these dialects have been observed are killer whales and sperm whales. Dialects in killer whales and sperm whales are believed to function as social identification markers of groups that continuously mix (Ford and Fisher 1982; Ford 1991; Weilgart and Whitehead 1997). However, there is a difference between the two types of groups, because killer whales appear not to disperse from their natal group while sperm whale group composition changes over time.

Studies of captive killer whales with different regional ancestry (Bain 1988; Ford, unpublished data) provide strong evidence that call dialects in these whales are vocally learned. This notion is further supported by the occurrence of true vocal mimicry and horizontal transmission of call structures among wild killer whales (Ford 1991; Deecke 1998). Deecke (1998) showed in his study on call structure differences of sister pods that progressive divergence takes place in one of the resident killer whale populations in

BC. However, the degree of call repertoire similarity between sister matrilines appears to be relatively stable over a period of at least 12 years.

Ford and colleagues have studied the social organization and vocalizations of resident killer whales off the coast of British Columbia for the last 25 years. This resident killer whale population consists of groups of closely related animals (matrilineal). Male or female pod members apparently show no dispersal from their natal group, and use specific dialects as vocal signatures of their pod (Bigg et al. 1987,1990; Ford et al. 1994a; Ford 1989,1991).

Ford (1991) suggested that the gradual process of pod fission resulting in the formation of new pods as suggested by Bigg et al. (1990) is accompanied by divergence of vocal repertoires. Newly formed sister pods that initially still spend a significant amount of time together would have the same repertoire of calls as their ancestral pod. Over time, because of copying errors of calls between generations and fewer contacts between sister pods, calls would change progressively and make the repertoires distinct. Therefore, he assumed that pods with very similar repertoires have split more recently than those pods that have fewer calls in common. According to this correlation of social and vocal divergence, all pods that share one or more call types also share recent common ancestors and are considered to constitute a *clan*.

Different clans have entirely distinct call-type repertoires. A resident killer whale community consists of pods, often from different *clans,* but a clan is always contained within one community. For example, the 'Northern Resident' community consists of three clans, called A-, G-, and R-clan, while only one clan, J-clan, forms the 'Southern

Resident' community. This led Ford (1991) to suggest that vocal clans reflect historical rather than current relations between pods.

The distinctions between killer whale clans appear to be developed and maintained through the process of cultural segregation or slow dialect separation. The goal of our study on acoustic and genetic differences between clans was to determine the biological consequences of the separation process. The possible biological functions of this process and their adaptive significance will be discussed.

Study Population

Killer whales are found in all oceans, but are most easily studied in protected inshore waters, such as those along the Northwest coast of North America. Beginning in the early 1970s, field studies on killer whales in these waters were undertaken to identify individuals and to determine population size, life history traits and social organization. These studies have revealed the existence of at least three non-associating killer whale populations in the Northeastern Pacific, *residents*, *transients*, and *offshores* (Bigg 1982; Leatherwood; et al. 1984a, 1984b, 1990; Bigg et al. 1987, 1990; Morton 1990; Olesiuk et al. 1990; Heise et al. 1991, 1993; Ford et al. 1994a, 1994b; Matkin & Saulitis 1994; Matkin et al. 1999). Although all three populations have overlapping summer ranges they appear to be socially and possibly reproductively isolated (Bigg et al. 1990; Ford et al. 1994a; Ford et al. in press; Barrett-Lennard, unpublished results.). Transients and residents also have different dietary preferences (Ford et al. 1998; Saulits et al. 2000).

Resident killer whales that feed exclusively on fish, mostly salmonids, can be regularly encountered in inshore waters ranging from Washington State to Alaska during the

summer months (Ford et al. 1998; Saulits et al. 2000; Ford et al. in press). Residents appear to stay in their natal groups, called *matrilines*, their entire life (Bigg et al.1990; Ford et al. 1994; Matkin et al. 1999; Ford et al. in press.). However, several related matrilines periodically form stable travelling groups called *pods*, which contain on average 10 to 25 animals but can exceed 50 animals (Bigg et al. 1987, 1990; Ford et al. 1994a). Residents from different pods meet regularly throughout the summer within a particular region (Fig. 1), and also form temporary larger aggregations. Those aggregations appear to serve a social function in mating, and in maintaining a cohesive community (Matkin et al. 1997). In Prince William Sound, most pods meet regularly during all summer months. However, in August, much larger aggregations of whales are often seen (Matkin et al. 1995).

Especially, during times when several pods meet an increased number of vocalizations are heard (Ford 1989). These vocalizations fall into three categories, *clicks*, *whistles*, and *calls*. *Clicks* are heard in 95% of all encounters with residents. They appear to be used by whales in the detection and pursuit of prey, as well as during social encounters (Barrett-Lennard et. al. 1996). *Whistles* are heard during social interactions when the whales are in close proximity to each other (Thomsen 1998). *Whistles* and *whistle*-like structures can be part of the dialects of residents; however, the most common type of vocalization that is used to describe dialects is the discrete *call* (Fig.2). *Calls* are highly repetitive and stereotyped pulsed vocalizations. They have distinct tonal properties because of high pulse repetition rates and many have whistle-like elements throughout parts of the call duration. The repetition frequencies of pulses are reflected in the harmonic contours seen in the spectrogram (see Watkins 1966), and these contours are usually modulated over the call's duration. Many calls contain silent intervals (Fig.3) as well as abrupt shifts in pulse repetition frequency (Fig.2) often accompanied by changes

in sound pressure, These intervals and shifts allow the call to be divided into different parts and elements. We called the aforementioned whistle-like elements, which are produced by higher repetition frequencies (higher harmonic bands in the spectrogram) upper frequency components, the ones with the lower repetition frequencies (lower harmonic bands) were called lower frequency components.

After echolocation clicks, calls are the predominant type of vocalization in the repertoire of resident killer whales. Discrete calls are heard in approximately 90% of all encounters, typically in situations where the whales are spread out foraging or when two or more pods meet. Ford (1989) suggested that the discrete calls of resident killer whales serve as signals for maintaining contact between matrilines or pod members. Each pod in British Columbia has a distinct set of 7-17 call-types. Each member of a pod is believed to learn and reproduce the entire repertoire of calls of its pod. Thus, pod specific dialects serve to identify a pod acoustically (Ford 1984, 1989, 1991). Ford (1991) also noted that call repertoires of some pods had remained relatively constant for more than 25 years.

Some calls appear as two or more stable variants. Those calls are referred to as sub-types of the same call-type (Ford 1984, 1987) (Fig.3). Deecke (1998) investigated the possible evolution of call variants and found a relationship of acoustic change and the degree of association between different matrilines. There are also calls that are not consistent in structure, which are referred to as aberrant calls (Ford 1989). These calls comprise 5% of all vocalizations, and are mainly heard when whales are in close proximity and are engaged in social interactions.

Methods

The procedure used to collect and analyze vocalizations was based on Ford (1984). Similar procedures have been applied to vocalizations of resident-type killer whales in Norway (Strager 1995), and to an isolated transient group of killer whales (AT1) in Prince William Sound, Alaska (Saulitis 1993).

Different observers made recordings parallel to photo-identification of the whales between 1984 and 1999 (Table 1). After the whales had been approached and photographed observers usually drove 500 metres ahead of the whales, and turned off their boat engines. A hydrophone was then lowered over the side of the boat to a depth of 10-15 metres.

The recording systems that were used varied between observers, but most often consisted of a Celesco BC-10 or BC-50 hyrdophone and a SONY WM-DC6 or MARANTZ PMD 221 cassette-tape recorder. The frequency responses of these recording systems was linear between 100 Hz and 8 kHz, and still relatively dynamic (± 10 dB) up to 14 kHz. Some observers used recording systems that had better frequency responses, such as a Bruel & Kjar (B&K) hydrophone, Type 8101, and a NAGRA IV-SJ reel-to-reel tape recorder or an Offshore Acoustics hydrophone and a TCD-D7 SONY DAT recorder. The frequency response of the NAGRA/B&K system was linear between 5Hz and 35 kHZ (± 1 dB) when the tape speed was set to 38.1 cm/s. The DAT/Offshore Acoustics system showed a linear response between 20Hz and 20 kHz (± 1dB). Recordings from the last two systems were preferably used for spectrographic analysis.

Recordings of each pod were analyzed only when the group was encountered alone or at such a distance from other whales that the calls could be attributed unequivocally to that group. Vocalizations were recorded during a wide range of observable behaviours, such as travelling (slow and fast), feeding, resting (milling at surface), and socializing (pod gatherings) as described by Bigg et al. (1990). It was impossible to only consider calls recorded in similar situations because of the great number of different observers and the resulting inconsistencies in describing behaviour contexts. Therefore, in order to avoid any wrong categorization of calls because of situation-related variation in call usage (Ford 1989), sub-types were only assigned when calls were consistently recorded in several different contexts.

All recordings meeting the above criteria were used to describe the call repertoire of a pod. Table 1 shows the number of existing recordings and the number of analyzed single pod recordings is displayed.

We classified call types acoustically by ear and visually by inspection of the sound spectrogram. Classifications were based on distinctive audible characteristics of the calls, which appeared as distinguishing structural differences in the frequency time contours of the calls' spectrogram. The method has been described previously by Ford & Fisher (1982) and Ford (1984). We used this method because Ford (1984) found no significant difference between the classification of killer whale calls based on a statistical comparison of certain sound parameters and the classification done by ear and visual inspection. Bain (1986) came to similar call categories using ear and visual inspection to classify calls from two captive killer whales from the same population that Ford (1984) analyzed. We gave call samples to two other researchers familiar with killer whale

vocalizations for re-classification. Categories reported here showed agreement among the majority of all classifications.

We inspected recordings for the presence of calls by using a Kay Elemetric DSP Sona-Graph, Model 5500, which allowed spectrographic real time signal representation. A sample of recognized calls (minimum of 15 per pod) were digitized and later analyzed using Canary, Version 1.2.4 (Cornell Laboratory of Ornithology 1998).

Distinct call types were named alphanumerically using the prefix AKS to designate that the calls were from Southern Alaskan killer whales. Numbers reflect the order in which the calls have been identified. There is no hierarchical structure within the numbering system. The appendices i, ii, iii etc. that are used in combination with some call types indicate the existence of sub-types (Fig. 3).

A quantitative measure of the similarity of call repertoires for each pair of pods was obtained from an index based on the degree of call sharing. This index was derived from Dice's coefficient of association (Morgan et al. 1976), which normalizes the data to account for differences in repertoire size:

$$\text{Index of Similarity} = \frac{2(N_C + N_S)}{R_1 + R_2}$$

where N_C is the total number of call types without variants shared, N_S is the total number of sub-types shared, and R_1 and R_2 are the repertoire sizes (call types plus subtypes) of the two pods. The index values ranging between 0 - 1 were then used to calculate a

hierarchical structure of acoustic similarity, displayed in the form of a dendrogram by means of single-link cluster analysis (Morgan et al. 1976).

Skin biopsies were collected for DNA analysis using lightweight darts projected with a pneumatic rifle (Barrett-Lennard et al. 1996). We approached whales slowly on a gradually-converging course, and then travelled parallel to them at a distance of 10 - 15-m. After taking identification photographs of the entire group, we selected a whale to biopsy that we could identify visually and that had not been biopsied previously. We fired the darts at a region of the back approximately 1 m behind the dorsal fin and 50 cm below the dorsal ridge. The darts were designed to excise and retain a 0.5 g plug of skin and blubber, and to bounce off the whale and float. We re-photographed the darted whale if possible to confirm its identity, and retrieved the darts from the water with a long-handled dip net. The number of biopsies that were collected from different pods each year is also presented in Table 1.

Results

The majority of the calls had more than one fundamental frequency (Fig.4). The lower fundamental frequencies ranged from 0.5 to 4.5 kHz (lower frequency component), while the upper fundamental frequencies ranged from 2 to 11 kHz (upper frequency component) (Fig.4). The energy distribution within the call spectrum usually allowed good spectrographic representation of frequencies from 0.5 kHz to 12-14 kHz. However, when call-to-noise ratios decreased fewer harmonics were visible in the spectrogram. Low call-to-noise ratios due to boat engine noise and other underwater sources selectively masked the upper frequency components of calls. As a result, we could not trace the upper frequency component when heavy background noise was present. Upper frequency components were also less likely to be seen in the spectrogram when the whales did not swim towards the hydrophone, or when they rapidly changed directions. On one occasion, we observed the disappearance of the upper frequency component in calls made by an animal during a sudden change of direction in front of the hydrophone.

Of the 9000 calls produced by the seven pods - AB, AD, AE, AI, AJ AK, and AN pod a total number of 38 discrete calls were identified in their vocalizations. These calls could be placed into 26 categories of distinct types. 10 of these 26 distinct types produced more than one stable variant or sub-type. Overall, one of the eight types appeared as 4 sub-types, one as 3 sub-types, and eight as 2 sub-types. Two examples of variations of the same call type are displayed in Figure 3. Spectrograms of all 26 distinct types are presented in Figure 4.

Calls such as AKS 17 and AKS 01 were structurally simpler, having fewer parts and elements and no upper frequency component. Simpler calls produced more sub-types than calls that were structurally more complex, such as AKS22 or AKS03. Generally, the appearance of sub-types indicated that these calls were shared between pods. However, there were two incidents in which sub-types existed in the repertoire of only one pod, AKS02 in AE pod and AKS24 in AJ pod. In both cases these pods shared the least amount of calls with any other member of the same *clan*.

The mean number of calls for each pod was 12.14 (s = 3.67), while the median was 13. Numbers ranged from 7 types in AK pod to 17 types in AB pod. Table 2 displays which calls have been used by which pod. The number of call types produced by a pod showed no correlation or trend relationship to the numbers of whales in that pod. For example, AB pod declined from 35 to 25 members during the study period while consistently using 17 calls. AJ pod increased from 25 to 38 members in the same period but was using 13 calls during the whole period. Similarly, the two AN sub-pods (AN10 and AN20), together consisting of approximately 50 whales were using 15 calls, while AI pod, which counted 7 members produced 14 calls. All types were recorded during times when the whales were reported displaying behaviour, such as *feeding, travelling, and socializing* with the exception of *resting* and *slow-travelling*. While resting the whales often did not vocalize or used particular call-types more than others. When slowly travelling the whales were mainly quiet. A detailed analysis of the acoustic behaviour of Alaskan resident pods will be reported elsewhere (Yurk et al. in prep.).

48% of all identified discrete calls were shared by more than one resident killer whale pod, and pods shared between 53 and 100% of their call repertoires with other pods. Calls that were shared are indicated by X-marks in the same row of Table 2. Although all

seven pods shared calls with at least two other pods the pattern of sharing revealed a distinction into two distinct clusters. AB, AI, AJ and AN pod shared calls, as did AD, AE, and AK pod, but no calls were shared between these groups. In use of the definition of Ford (1991) we considered pods that shared calls to belong to the same acoustic *clan*. Accordingly, we considered AB, AI, AJ, and AN pod belonging to *AB-clan*, and AD, AE, and AK pod belonging *to AD-clan*.

Call variants or sub-types were only recorded from pods of the same *clan*. Overall, more sub-types were shared than calls without variants, and the maximum number of sub-types of a particular call correlated with the number of pods pertaining to that *clan*, which was four in *AB-clan* and three in *AD-clan*. *AB-clan* used a mean number of 14.25 calls (s=1.71) while *AD-clan* used a mean number of 8.67 calls (s=2.08). Occasionally, contour-distorted versions of a call type were recorded. These versions were considered call-mimics because they were produced by members of a pod that was in acoustical proximity of another pod that regularly produced the non-distorted call-type.

We calculated the degree of repertoire similarity among pairs of pods of each *clan* separately using the acoustic similarity index. Because pods from different *clans* did not share any calls the acoustic similarity between them was 0 (Table 3). The repertoires of AB, AI, and AN pod within *AB-clan* and AD and AK pod within *AD-clan* are very similar in comparison to either of the repertoires of AJ and AE pod in their respective *clans*. The results of the repertoire analysis is displayed in the form of a dendrogram by means of single-link cluster analysis (Morgan et al. 1976) (Fig. 5).

Based on a sequence analysis of the entire D-loop region of the mitochondrial DNA, we could detect maternal relatedness between matrilines and pods. The four pods that belong to *AB-clan* showed the same mitochondrial haplotype (Fig.5). This haplotype has been found in all biopsied killer whales of the Northern Resident (NR) community. This community comprises killer whales known from British Columbia and Southeast Alaska. In contrast the pods of *AD-clan* all showed a mitochondrial haplotype that has been found in whales of the Southern Resident (SR) community (Fig.5). Killer whales of this community can usually be found in Southern British Columbia, Washington State and occasionally further south.

Discussion

It remains controversial whether vocally learned dialects could be an advantageous adaptation and play a role in the gene flow within and between populations (Baptista 1975; Treisman 1978; Slater and Ince 1979; Conner 1982; Mundinger 1980, 1982; Baker and Cunningham 1985; Baker and Jenkins 1987; Ford 1991; Slater and Williams 1992; Catchpole 1996; Lynch 1996:p.181; Payne 1996:p.198). However, the main problem might be that vocal variations that are caused by geographic isolation should not be called dialects, because they do not have a social function. Social dialects that occur within and between breeding populations might however be important restrictions for the distribution of genes. Although many social dialects between populations that are in close proximity result from cultural mutation and drift (neutral evolution) (Slater and Ince 1979; Payne 1985; Williams and Slater 1992; Payne 1996) this does not explain the prevalence and long stability of dialects among mixing populations (Ford 1991; Wenrick Boughman 1997; Weilgart and Whitehead 1997). In these situations, social dialects may function as a possible reinforcement in assortative mating to avoid inbreeding or

outbreeding depression (Treisman 1978; Krebs and Kroodsma 1980; Mundinger 1982; Baker and Cunningham 1985).

Our results point to a co-evolutionary process between call repertoire stability and female relatedness. Residents acquire their dialect within the maternal unit through copying selectively only calls of their close kin, while not incorporating calls of whales from other groups, which they are exposed to regularly. As a result, dialects of residents become very stable, e.g. the repertoire of one pod of the Southern Resident community has not changed considerably during the last 50 years (Ford, pers, comm.).

Following Ford (1991) we called pods that share parts of their call repertoires members of the same acoustic *clan*. In our analysis of the mitochondrial DNA we found that AB-clan individuals share the same D-loop sequence, which differed from the sequence shown by all AD-clan members. This indicates that there has been no effective dispersal of females between clans since the time that the split occurred between pods in Alaska and those in British Columbia and Washington State (Fig 5). This is further supported by no observed exchange of individuals between matrilines in either Alaska (Matkin et al. 1999) nor British Columbia/Washington State (Bigg et al. 1990; Ford et al. 2000).

(Ford 1989) suggested that dialects function in maintaining cohesion within and between social groups that associate often. Therefore dialects could play a significant role in maintaining the cultural and physical segregation of pods along the lineages of relatedness.

Alaskan resident killer whale pods as well as their counterparts further south have been observed to mix often with other pods during the summer months. During these

gatherings mating is commonly thought to take place (Bigg et al. 1990), although sexual behaviour at the surface has only been observed between members of the same sex (Rose 19??). Matings most likely occur underwater, because males or females from one pod have been observed swimming with a different pod for periods of time. Calls, particularly discrete calls, are the predominant types of vocalizations heard during these social gatherings. A possible function could be sexual advertisement used either by males alone or by both females and males. Increased call rates during these social interactions in comparison to other behaviours appear to reflect elevated arousal levels of the animals. (Ford 1989; Yurk, unpublished data.). Therefore, whales could choose mating partners according to the discrete calls they use, both as an advertisement of health and as an indicator of cultural and genetic lineage.

Barrett-Lennard (in prep.) compared microsattelite DNA among residents, and showed that the degree of relatedness among A-clan members of the Northern Residents in British Columbia decreases proportionally to the decrease in acoustic similarity detected by Ford (1991). This indicates that residents mate with whales that are acoustically dissimilar. This type of negative assortative mating over time tends to homogenize populations genetically. Because dialects in free ranging whales are culturally inherited from related adults (Ford 1984, 1989, 1991), as well as related members of the same generation (Deecke 1998) female residents might counteract homogenizing by creating stable cultural lineages of related pods. The whales can use these lineages to determine the degree of relatedness of a possible mate to decrease inbreeding risks, and through non-dispersing from natal groups avoid genetic homogenization. The easiest way to create cultural lineages, is by learning dialects as distinct acoustic elements. This would mean that repertoires as well as single calls should be structured as distinct elements. Calls could then remain similar and reflect relatedness long after pods have separated,

while the loss or replacement of complete elements would create more distinct repertoires among pods that travel consistently alone. This will over time make pods more distinguishable without loosing acoustic similarity altogether.

There is evidence for element structure in calls and repertoires of residents (Ford 1984; Bain 1988; Deecke 1998; Yurk unpublished data). Deecke (1998) for example found that a call type with higher structural complexity did not change considerably over a period of twelve years, while a type with lower structural complexity changed notably during the same period. While using artificial neural networks to compare frequency contours of calls, he demonstrated that the frequency contours of both calls of associating resident killer whale sub-pods changed structurally over a 12-year period in a parallel fashion. At the end of the 12-year period, call structures were more similar between calls of different sub-pods than they were between the sub-pod's own calls at the beginning and the end of the 12- year period. However, the progressive changes that occurred during the 12-year period did not make the calls so distinct that they could be considered different call-types defined by Ford (1984, 1987) and in this study. This might be because they did not affect their element structure. The calls that showed a lower degree of differentiation (fewer elements) changed more than calls that were structurally more complex (several elements) (Deecke, 1998). The low or non-existing variation in one of the call types suggests a more selective way of transmitting calls rather than a random effect caused by cultural drift. There is further evidence for structural coherence in calls caused by cultural selection among call types that appears to be strictly based on discrete element variation (Ford 1984, Yurk, unpublished data).

Cultural selection of useful information for adapting quickly to changing environments is common among humans (Cavalli-Sforza 1988) and other primates (Nishida 1986). Hill

(1978) was the first to explore how dialects could have influenced the distribution of genes among human populations by creating a model for human dialect evolution. Hill suggested that dialects, which occurred through copying errors during the vocal learning process, reinforce separation and promote endogamy in groups or communities that are otherwise culturally distinct. Eventually, this process leads to local *demes* or small populations that share the same genetic and cultural heritage. Based on this model, dialects, which form within a single human language group provide the precursors of new languages. This model could also explain in the most parsimonious way the evolution of language families (Barbujani (1991?); Cavalli-Sforza 1991; Ruhlen 1994). Language divergence appears to be accompanied by other important cultural traits. Language differences in connection with farming techniques appeared to have fostered monopolization of food resources by limiting marriage within a particular community (Renfrew 1989). A similar reason for strong cultural selection for segregation could be assumed for residents. Residents have acquired particular foraging techniques well adapted for inshore foraging, particularly on salmonids (Barrett-Lennard et al. 1996; Nichol and Shackelton 1996; Ford et al., 1998; Saulitis et al., 2000) The current distribution of residents is closely tied to the distribution of returning salmonids along the Pacific coast. They live sympatrically with other killer whale populations (transients and offshores) yet maintain a geographic boundary between resident communities during their main feeding periods on salmonids.

In order for resident killer whales to monopolize food resources, such as different salmonid runs effectively, the development of cultural traditions may be very advantageous. The social structure of the resident community is matriarchial. Females outlive males by an average of 20 years (Olesiuk et al. 1990). Most females become post-reproductive when they are around 45 years, and longevity of some females

reaches 90 years and more. The four different salmon species that spawn in rivers off the West coast return at different places at different times throughout the summer and fall. Knowledge of space and time of the salmon return pattern is best kept within a group of whales by a long living adult. Cultural traditions such as dialects minimize the risk that this knowledge will leave the community of related whales.

Nichol and Shackelton (1996) found particular resident pods to be correlated with the availability of certain salmon prey species. Ford et al. (1998) discovered that chinook and sockeye salmon were the preferred prey of residents. The majority of the sample that Ford et al. (1998) analyzed were primarely from A-clan pods. These whales were observed eating chinook and sockeye in Johnstone Strait, British Columbia more often than whales from the two other clans of the Northern Residents. A-clan possibly monopolizes chinook and sockeye prey in Johnstone Strait during a particular time of the year.

We suggest that the dialects of resident killer whales are culturally selected traditions to reduce competition between clans by monopolizing times and areas of increased food abundance. Larger, and possibly older, clans such as A-clan of the Northern residents and AB-clan of the Alaskan residents contain more matrilines. They may monopolize core foraging areas while smaller and possibly younger clans have to feed more often in more peripheral areas. Mating, which appears to take place later in the summer (Bigg et al. 1990) makes aggregations of matrilines necessary. Dialects appear to be used to avoid higher levels of inbreeding by selecting mates that are acoustically very dissimilar. The resident population possibly evolved by cultural segregation and behavioural character displacement (Barrett-Lennard 1999). Cultural segregation was achieved through retaining knowledge that allows the effective use of the spatially and temporally predictable abundance of an energy rich food source, the salmon.

Acknowledgements

We would like to thank the many observers who made recordings. In particular, we would like to thank Eva Saulitis and Kathy Heise, whose recordings made a substantial difference to the amount of data that could be used. We also would like to thank the Oil Spill Recover Institute and the *Exxon Valdez* Oil Spill Trustee Council for providing funding for parts of this study. We would like to thank the Vancouver Aquarium Marine Science Centre for letting us use their Acoustic Laboratory, and the BC Wild Killer Whale Adoption Program for providing financial support for one of the authors. We would like to thank Kathy Heise for reviewing earlier drafts of this manuscript and providing very helpful comments.

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Table1:

Year/Pod	*AB*	*AI*	*AN*	*AE*	*AK*	*AD*	*# of recs./ year*	
1984	9	3	4	3	2	4	25	(22.3%)
1985	4	0	4	4	1	2	15	(13.4%)
1986	0	0	1	1	0	1	3	(2.7%)
1988	0	0	0	0	1	0	1	(0.9%)
1989	2	0	1	0	3	0	6	(5.3%)
1990	1	3	2	2	2	1	11	(9.8%)
1991	0	3	2	3	4	1	13	(11.6%)
1992	3	2	1	2	1	0	9	(8%)
1993	0	0	0	1	0	1	2	(1.8%)
1994	0	1	0	0	0	0	1	(0.9%)
1996	1	2	0	3	0	0	6	(5.4%)
1997	0	2	5	3	2	4	16	(14.3%)
1998	1	0	0	0	1	1	3	(2.7%)
1999	0	0	0	0	0	1	1	(0.9%)
total # of recordings	21	16	20	22	17	16	112	
total # of biopsies	8	2	6	5	3	4	28	

Table 2

	AB-Pod	AI-Pod	AJ-Pod	AN*-Pod	AD-Pod	AE-Pod	AK-Pod
AKS 01 i					X		X
ii					X(AD16)		X
iii					X(AD5)		
AKS 02 i						X	
ii						X	
AKS 03					X	X	X
AKS 04 i					X	X	
ii					X	X	X
AKS 05					X	X	X
AKS 06						X	
AKS 07	X	X	X	X			
AKS 08 i	X	X		X			
ii	X						
AKS 09 i					X	X	X
ii					X		X
AKS 10 i	X		X	X			
ii	X	X	X	X			
AKS 11 i	X	X	X				
ii	X	X	X	X			
AKS 12	X						
AKS 13	X	X		X			
AKS 14	X	X		X			
AKS 15 i	X	X	X	X			
ii	X	X	X	X			
AKS 16				X			
AKS 17 i	X	X		X			
ii	X	X					
iii	X	X		X			
iv				X			
AKS 18					X (AD5)		
AKS 20				X			
AKS 21					X (AD16)		
AKS 22	X	X		X			
AKS 23			X				
AKS 24 i			X				
ii			X				
AKS 25	X	X	X				
AKS 27			X				
AKS 28			X				
TOTAL	17	14	13	15	11	8	7

Table 3

AB							
AD	0						
AE	0	0.444					
AI	0.903	0	0				
AJ	0.533	0	0	0.519			
AK	0	0.824	0.533	0	0		
AN	0.8	0	0	0.815	0.522	0	
	AB	AD	AE	AI	AJ	AK	AN

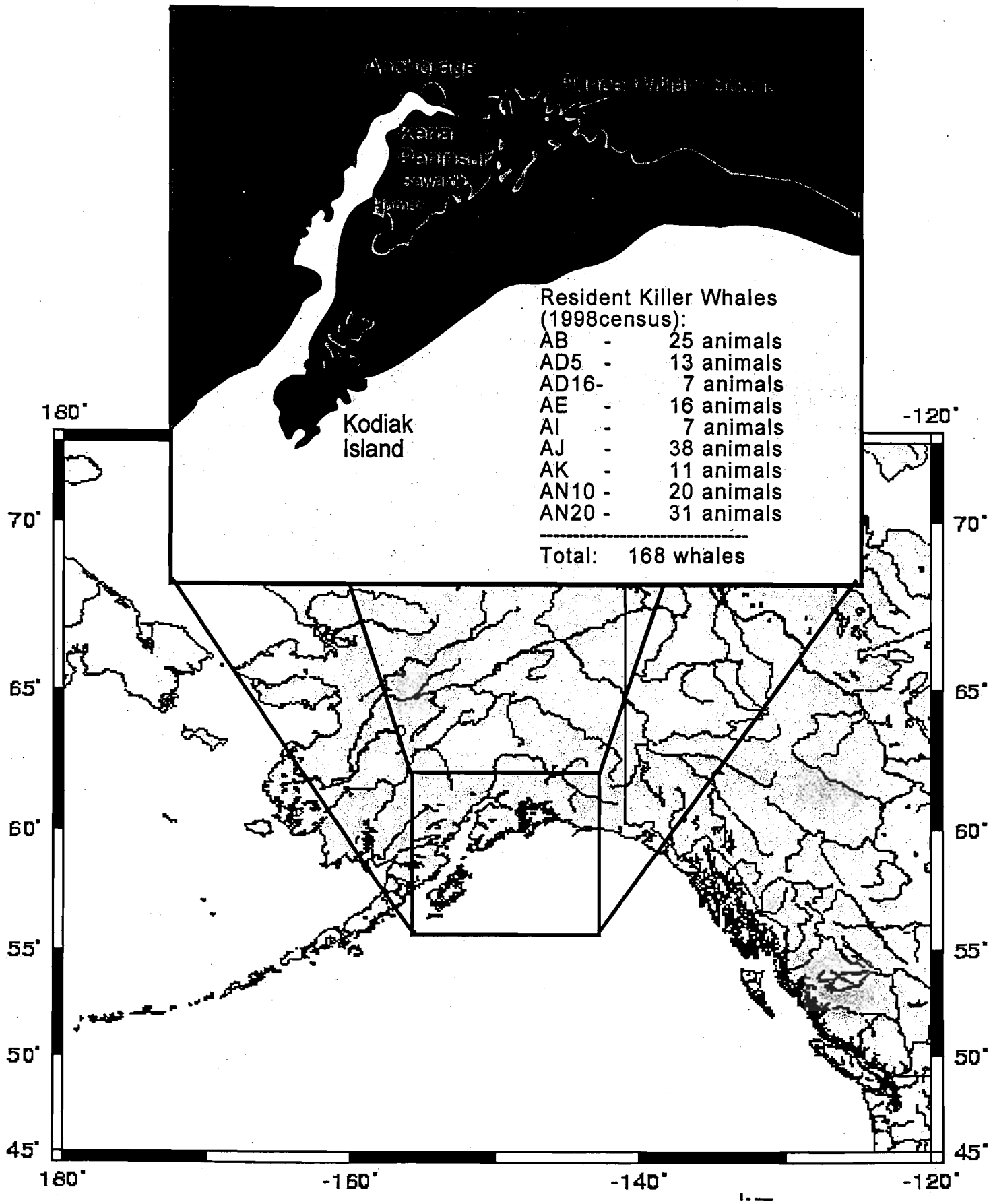

Kodiak
Island
Resident Killer Whales
(1998census):
AB - 25 animals
AD5 - 13 animals
AD16- 7 animals
AE - 16 animals
AI - 7 animals
AJ - 38 animals
AK - 11 animals
AN10 - 20 animals
AN20 - 31 animals
Total: 168 whales
180°
-120°
70°
65°
60°
55°
50°
45°
180°
-160°
-140°
-120°

Figure 1

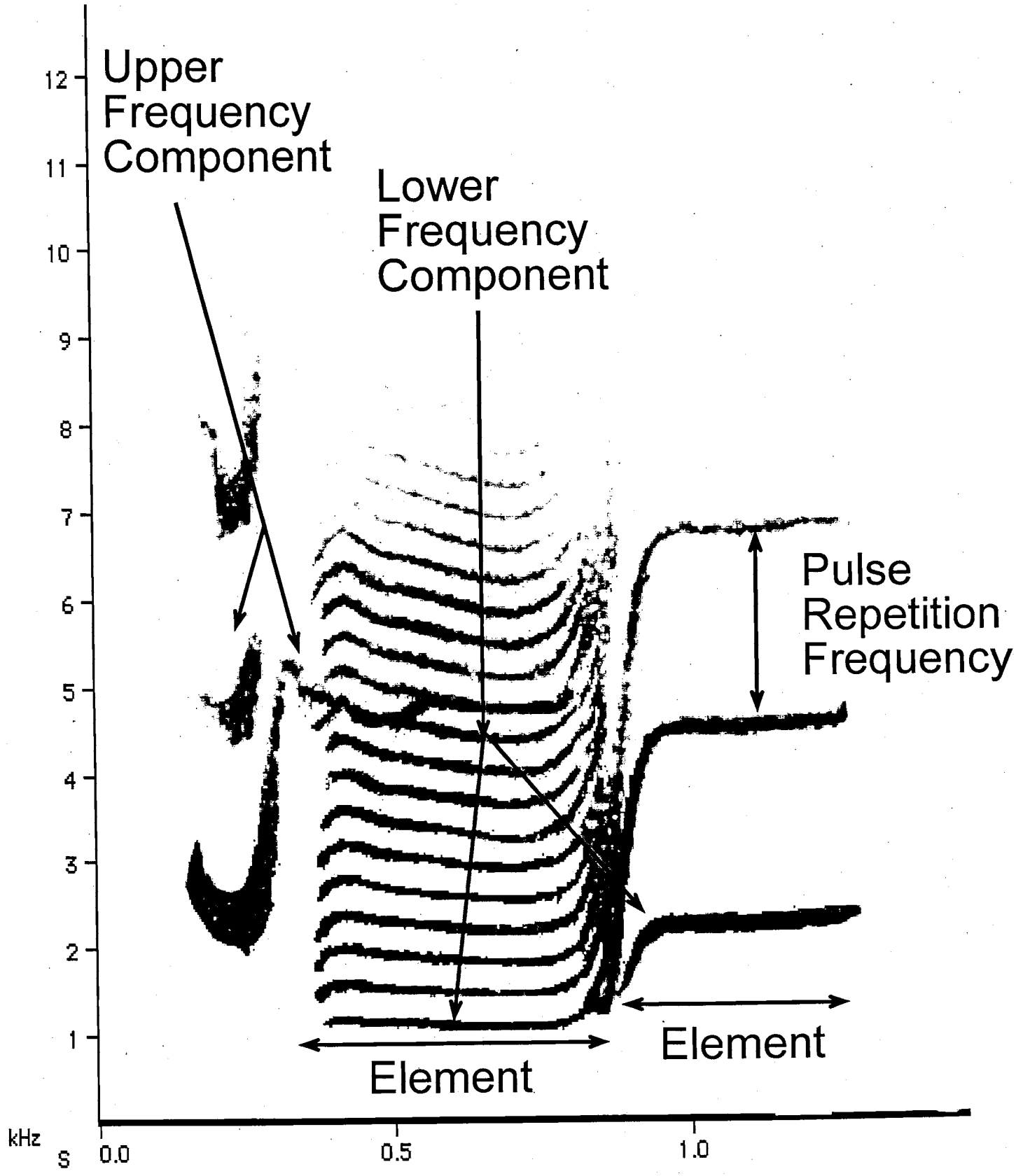

Upper
Frequency
Component
Lower
Frequency
Component
Pulse
Repetition
Frequency
Element
Element
12
11
10
9
8
7
6
5
4
3
2
1
kHz
S
0.0
0.5
1.0

Figure 2

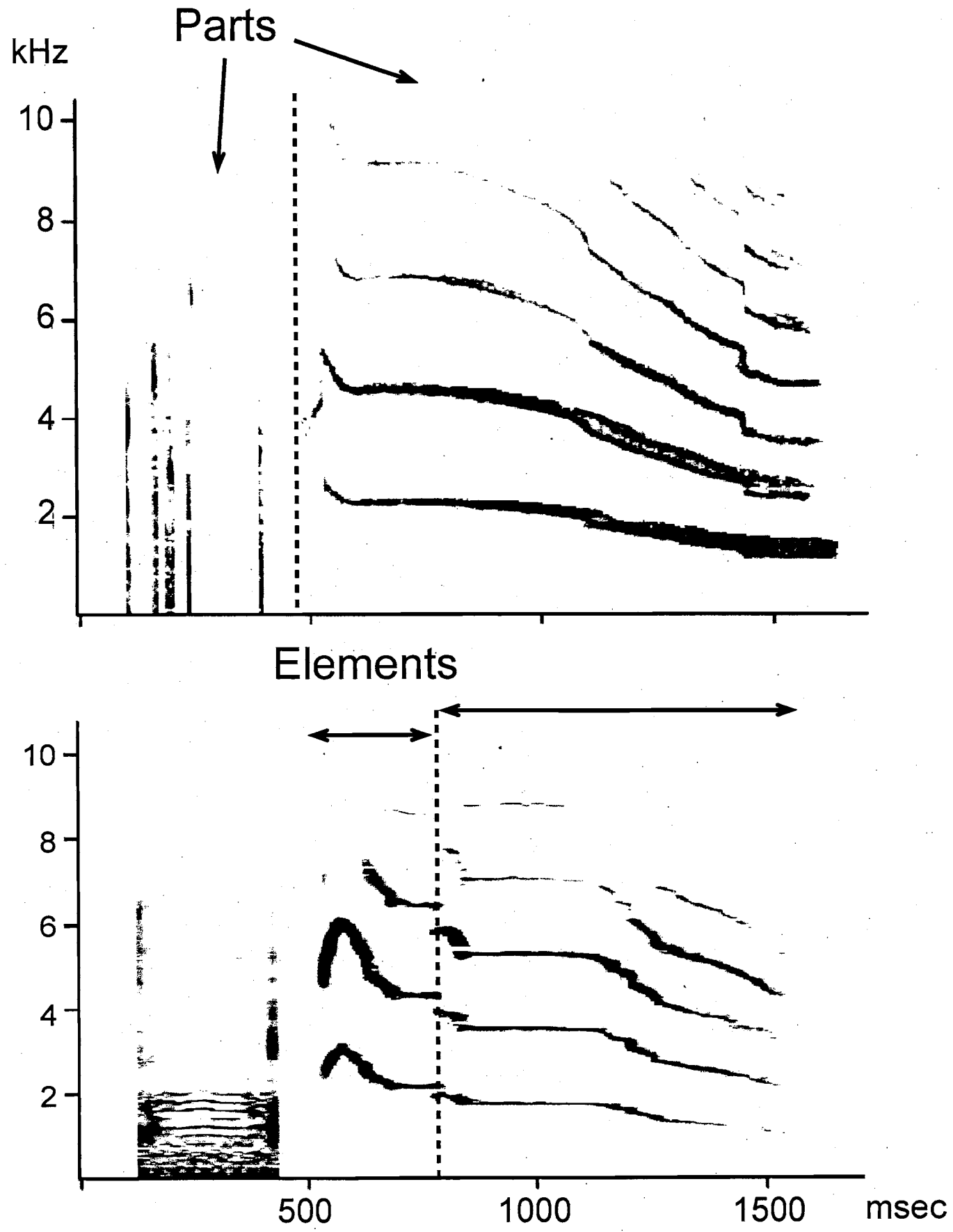
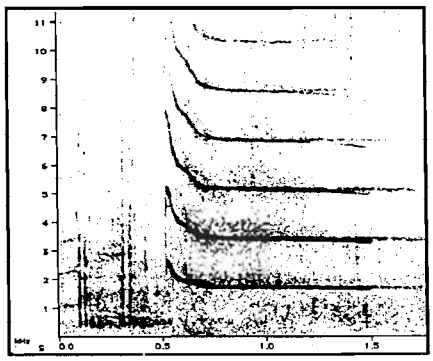


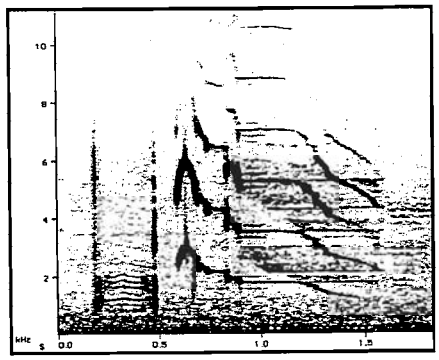
Figure 3

AKS01

AD5



AD16



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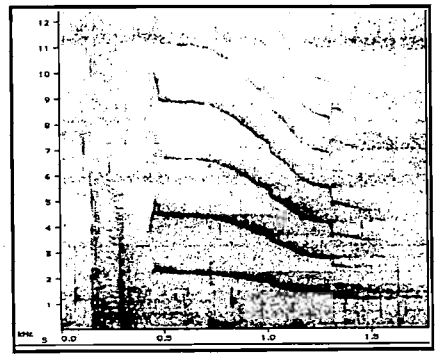

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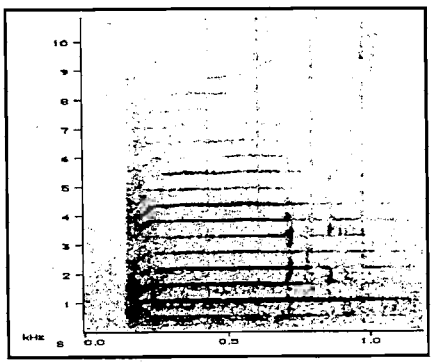

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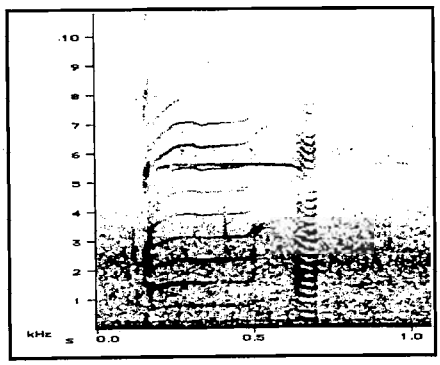

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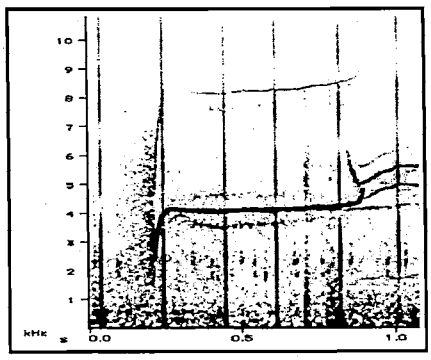

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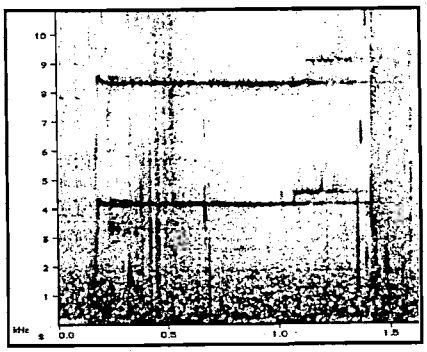

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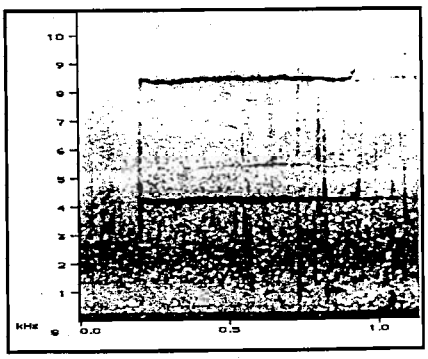

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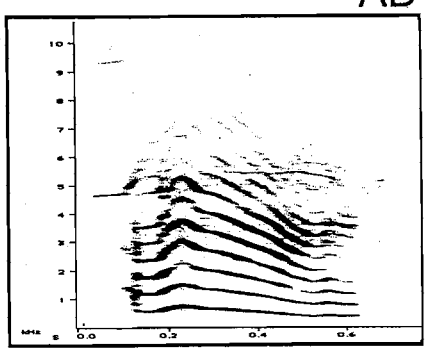

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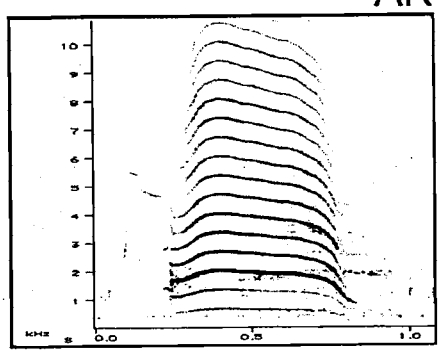

Figure 4a

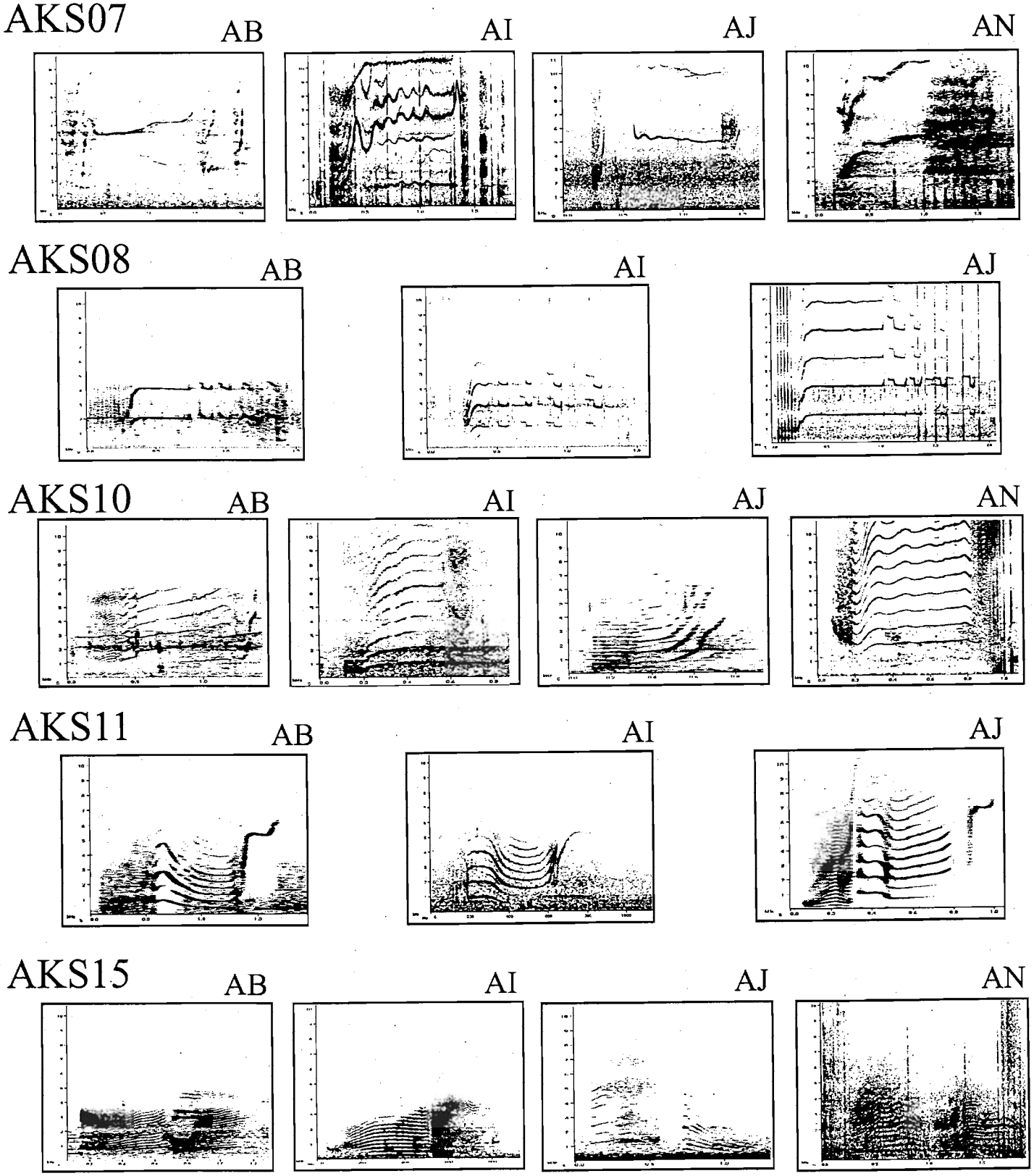
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FIGURE 4b

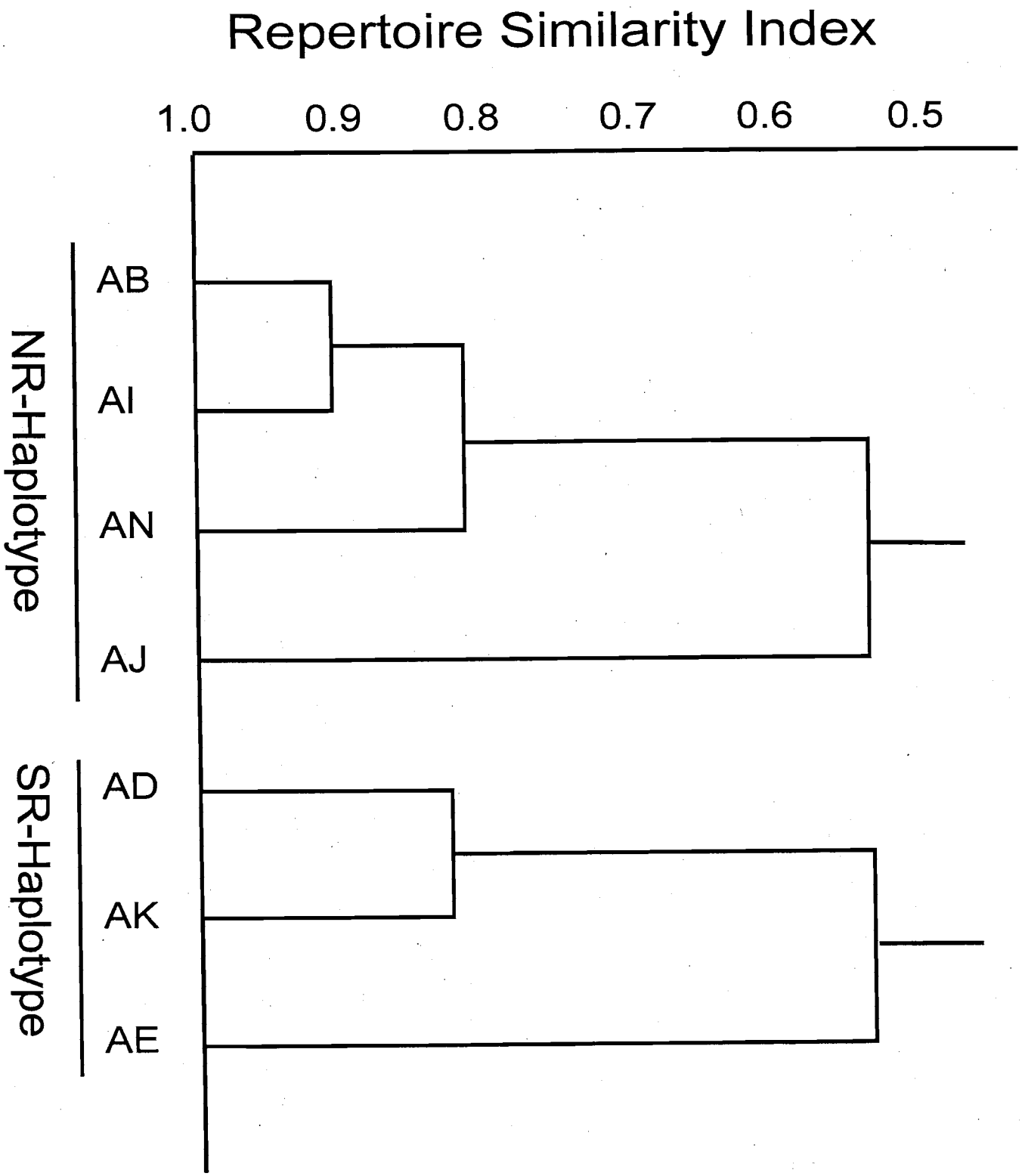
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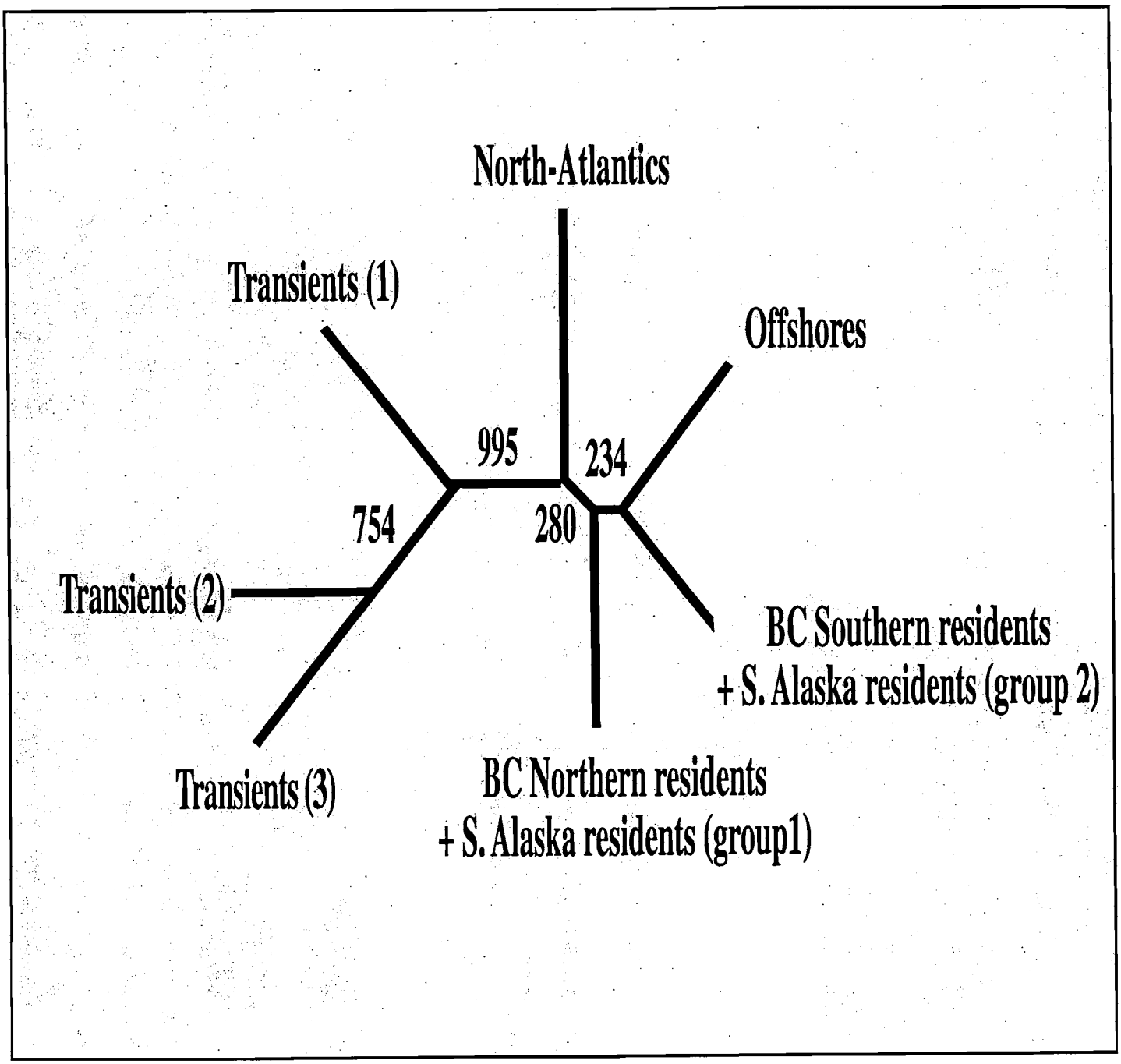
Figure 5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Transients (1)
Offshores
995
234
754
280
Transients (2)
BC Southern residents
+ S. Alaska residents (group 2)
Transients (3)
BC Northern residents
+ S. Alaska residents (group1)

Figure 6