Exxon Valdez Oil Spill
Restoration Project

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

Restoration Project 030012
Final Report

This final 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.

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March 2003

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STUDY HISTORY: The current project was initiated under Restoration Project 95012 "Comprehensive Killer Whale Investigations" and became "Photographic and Acoustic Monitoring of Killer Whales" in 1999. This is the final report for both studies although continued monitoring work is expected. Prior to the current year's 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 spring 1997, 1998, 1999, 2000, 2001 and 2002. 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), feeding habits (Saulitis et al 2000), habitat use (Scheel et al 2001) and contaminant levels (Ylitalo 2002) have been published and are included, at least in part in this report. This report also contains results of field work supported by the Alaska Sea Life Center in 2002.

ABSTRACT: Killer whale research was initiated in 1995 and included population monitoring and modeling, genetic and acoustic analysis, examination of distribution and movements, and an examination of feeding habits. At the conclusion of this study, 13 years after the spill, the damaged AB pod numbered 26 whales, and had not recovered to the prespill number of 36. The AT1 transient group lost nine of 22 whales following the spill and has not recruited a calf since 1984. Genetic analysis indicated AB pod was part of a larger southern Alaska resident population, however, the AT1 transients are genetically unique. Their decline is attributed not only to the *ExxonValdez* oil spill but possibly to the high levels of contaminants (PCBs and DDTs) in their blubber as well as a region-wide continuing decline in numbers of harbor seals (a primary prey), and the genetic/social isolation of the group. The AT1 transients consumed primarily harbor seals and Dall's porpoise and due to bioaccumulation have some of the highest PCB and DDT levels recorded for any mammal. Two acoustic clans of resident killer whales are found in Kenai Fjords/Prince William Sound and are confirmed by mt DNA haplotypes. Predation by resident whales was solely on fish and focused on Chinook and coho salmon. Pods and populations can be separated acoustically making identification of whales via remote hydrophone possible during winter months. GIS based analysis demonstrated habitat use differences between resident and transient populations and some range separation between pods.

KEY WORDS: acoustics, biopsy, contaminants, *Exxon Valdez*, Geographic Information System, feeding habits, foraging, genetics, killer whales, photo-identification, populations, *Orcinus orca*, Prince William Sound, Kenai Fjords, resident, transient.

PROJECT DATA: Identification data 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), 60920 Mary Allen Ave., Homer, Alaska 99603, (907) 235-6590. All field observations, killer whale encounter data, vessel logs and tracklines are stored in a GIS system (Arc/Info) housed at Alaska Pacific University, Anchorage, Alaska (Contact David Scheel) or at U.S. Fish and Wildlife Service, Marine Mammals Management, 1011 Tudor Rd, Anchorage, Alaska (Contact Doug Burn). This data is now available for inspection and use with permission of NGOS.

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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 1995-2002 as part of the EVOS Trustee Council restoration program reported here. 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 comprehensive program initiated in 1995 that evolved into a monitoring program in 1999 and continued into 2002. The goal of the annual photo-monitoring that has been part of the project from its inception has been to obtain identification photographs of all whales in all major resident pods and in 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 1984-2002 and is used to provide individual identifications for each encounter with whales. Vital rates for AB pod and all other frequently sighted resident pods have been calculated annually based on the photographic data.

The total number of whales in the seven well-known resident pods other than AB pod has increased from 81 to 125 whales from 1988 through 2002, while AB pod has declined from 36 whales to 26 whales in that same time period. Population modeling has demonstrated an intrinsic annual rate of increase of 3.3% for all pods other than AB pod through 2002. From 1995 to 2001, AB pod has had a net increase of four individuals, due to recruitment of nine calves and five mortalities. Eight members of the pod (AB25 subpod) still appear to travel with AJ pod a majority of the time, although they maintain their AB pod vocal dialect. Recruitment rates for AB pod now meet or exceed those of other pods and there are nine reproductive females in the pod, but recovery has been hindered by unexpected mortalities. However, the primary reason for lack of recovery of AB pod has been the disproportionate loss of reproductive and juvenile females at the time of the spill, resulting in a reduction of the reproductive potential within the pod.

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 13 of the 22 whales in the AT1 group have died since the spill. Nine of these whales died during the year of the spill. Three of the missing whales (AT1, AT10, AT19) were stranded and are known dead.

In our genetic studies, skin biopsies were obtained from 269 identified individual whales from our study area as well as from British Columbia. Nuclear DNA from the samples was typed at 11 polymorphic microsatellite loci, and the entire mitochondrial DNA control region was sequenced. The results have the following implications: (1) resident and transient killer whales are reproductively isolated, (2) both are subdivided into regional subpopulations between which migration is restricted, (3) the two ecotypes are reciprocally monophyletic, implying that they diverged once, (4) residents remain within their natal pods for life and have significantly lower levels of genetic diversity than transients, and (5) AB pod is part of a larger southern Alaska resident population and the of "AB clan". (6) The AT1 transients are a genetically unique population reproductively isolated from other transient populations. The study suggests that killer whales have a propensity to restrict association and mating to a community of several hundred individuals or fewer, and that this tendency is sufficiently strong to allow sympatric populations to maintain fixed genetic differences and, presumably, to speciate sympatrically.

Our acoustic analysis has indicated at least two acoustically and genetically distinct clans of resident killer whales inhabit Prince William Sound, Alaska called AB and AD, with no evidence of

sharing of call types between clans. It thus appears that the acoustic differences between the clans, which we presume to be cultural, reflect a genetic distinction between them. This means that there has been no effective dispersal of pods between clans, and presumably no effective dispersal of females from their natal group, the matriline. Further, we found differences in the degree of repertoire similarity between pods within each clan. Each resident pod and transient population can be separated by call repertoire and the call catalogue developed from this study can be used to identify killer whales recorded by remote hydrophones.

Remote hydrophone recordings have been used during the winter months to identify specific resident pods using Kenai Fjords/ Resurrection Bay. It has proven effective in determining patterns of use for resident pods when field operations would be impossible and expensive. In recent years the November-March period recordings are primarily of AB clan whales (including AB, AJ, and AN pods) with AD clan whales becoming predominant by April The area is used year round by resident killer whales.

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 transient, 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 Coupled with subsistence hunting (350+ seals per year), predation by killer whales could have a significant impact and inhibit the recovery of harbor seals. Current observations of healthy pups and low recruitment rates for harbor seals would seem to support this hypothesis (K. Frost, pers. comm.). Resident killer whales do not eat marine mammals and appear to select coho salmon from mixed schools during the July to September period (Saulitis, et.al. 2000) while Chinook salmon are selected in the April to June period.

Thirteen years of encounter data (1984 - 1996) were used to examine killer whale distribution within Prince William Sound, Alaska. Four patterns of area use were found, which comprised differences between resident and transient whales and differences among resident pods. Resident pods frequented large open passages, while transient groups used the narrow passages and bays in the southwest. This dichotomy likely reflects resident use of salmon and transient use of pinniped prey resources, as well as the different foraging strategies required for these prey types. Four resident pods of the same acoustic clan, AB, AI, AJ, and AN, used Knight Island Passage more than other areas of the Sound; two pods,AE and AK, used all areas of the Sound more evenly. Use of the Sound by the AT1 transient whales declined in the latter part of the study. Nearshore foraging for pinniped prey by the AT1 transient whales was more common in areas where these whales spend a disproportionate amount of time, suggesting that these areas were critical foraging habitat for the whales. Near shore foraging was directed at harbor seal prey No similar pattern emerged for Open-water Foraging for cetaceans by AT1 whales, nor for foraging by the resident whales.

Biopsy blubber samples of free-ranging resident and transient killer whales were acquired during the 1994 – 1999 field seasons and analyzed for selected organochlorines (OCs), including dioxin-like CB congeners and DDTs. Concentrations of OCs in transient killer whales (marine mammal-eating) were much higher than those found in resident animals (fish-eating) apparently due to differences in diets of these two killer whale eco-types. Reproductive female whales contained much lower levels of OCs than sexually immature whales or mature male animals in the same age class likely due to transfer of

OCs from the female to her offspring during gestation and lactation. Recruitment order also influenced the concentrations of OCs in the Alaskan killer whales. In adult male residents, first-recruited whales contained much higher OC concentrations than those measured in non-first-recruited (e.g., second recruited, third recruited) resident animals in the same age group. Contaminants may play a role in the non-recovery of the AT1 transient population.

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 were 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 18 years for the other resident pods in Prince William Sound or over the past 24 years for 19 resident pods in British Columbia and Washington State (Ford, et. al. 1982, Bigg 2000, Olesiuk, et. al. 1990, Matkin, et. al. 1999c). Following the spill, the social structure within AB pod demonstrated signs of deterioration. Subgroups traveled independently of the pod, and pod members did not consistently travel with closest relatives. The pod was observed less often; prior to the spill, AB pod was the most frequently encountered resident pod in Prince William Sound (Matkin, et. al. 1994). This study examines the recent population parameters for AB pod compares the pod with the rest of the resident population.

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 States. 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.

While mortalities in transient groups cannot be confirmed with the same certainty as for residents, AT1 transients have not been observed in adjacent regions, and in light of sighting records prior to the spill, it is extremely likely the missing individuals in the AT1 transients are dead. Most of the mortalities occurred in the year following the spill

The AB pod and AT1 group appear to have been injured due to the effects of the *Exxon Valdez* oil spill and neither has recovered although a slow recovery of AB pod may be occuring. In the course of our research that focused on these whales we have also collected acoustic data, samples for

genetic and contaminant analysis, remains of prey items, and killer whale movement and distribution data. Here we present the analysis and results of these field efforts in the appropriate sections of this report.

The genetic analysis used both mitochondrial DNA (mtDNA) and nuclear DNA microsatellites 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 (see Genetics).

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 make possible identification and enumeration of whale pods and groups from calls collected via remote hydrophone stations.

We have developed both our observational expertise and sampling methods for identifying prey of both resident and transient killer whales. In addition we have examined stomachs of dead, stranded whales. Although it takes many years of such observation and sampling to develop and understanding of feeding habits, for some populations, particularly the southern Alaska residents and AT1 transients, we have we have a basic picture of feeding habits, at least during the summer months.

A geographic information system (GIS) database was designed and the data from 1984 to 2002 has been 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/Kenai Fjords. Data analysis has provided demographics and spatial distributions of resident and transient killer whales detailed in this report.

Contaminant analysis has been completed on blubber tissue collected simultaneously with the genetic samples. The National Marine Fisheries Service Environmental Contaminant Laboratory in Seattle, Washington conducted the analysis 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. They are comparable or exceed levels in other marine mammal populations believed to have been negatively impacted by contaminants.

OBJECTIVES

1. To determine effects of the *Exxon Valdez* oil spill on numbers of killer whales in Prince William Sound/Kenai Fjords region

2. To monitor recovery of pods /populations that declined at the time of the spill using photoidentification techniques.

3. To determine feeding habits of resident and transient killer whales in the region

4. To use genetic and acoustic techniques to determine population structure of killer whales in the region.

5. To use GIS techniques to examine distribution/movement patterns for pods/populations and identify important killer whale habitat

6. To develop remote hydrophone systems and collect killer whale dialect data in order to track pods and populations remotely during the winter months.

7. To develop baseline contaminant level data for known individual whales and determine possible effects of these contaminants on the growth and recovery of killer whale pods/populations

FIELD METHODOLOGY

Fieldwork for the entire study period was completed from small vessels (less than 12 meters). The primary research vessel for the past three years has been *R/V Natoa,* a 10.3 m inboard diesel powered vessel, capable of 18 knots and sleeping 4 researchers. In addition a number other vessels have been used over the years, all have been smaller than 10 meters and have had either outboard on diesel inboard engines. These vessels operated in both the Kenai Fjords and Prince William Sound region. In addition during most years, 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 encounters sheets during surveys in Prince William Sound primarily directed at humpback whale photo-identification. The daily vessel logs and killer whale encounter sheets for this vessel were included in the GIS database and used in our analysis.

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. In Kenai Fjords, Channel 77 was also monitored. 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 Fuji Neopan 1600 high-speed black and white film. A Nikon N70 or similar auto focus camera with internal motor drive and a 300mm f4.5 auto focus 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 (Scheel et al 2001). On these logs, the elapsed time and distance traveled were recorded. Vessel track was plotted and 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 2-5 hours, providing considerable behavioral information (travel rates, duration of feeding bouts, etc.).

Directed observations of feeding behavior and identification and collection of killer whale prey were made when possible during the 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 lob tailing 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 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. 10cm 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 ml cryovial containing 1. 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 lowered over the side of the vessel 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 recorder showed a flat response to signals up to 15 kHz.

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 have declined following the *Exxon Valdez* oil spill. This project continued using photo-identification to monitor changes in resident killer whale pods and groups including AB pod and the AT1 transient group in Prince William Sound/Kenai Fjords.

Methods

Photographic Analysis

All photographic negatives collected during the fieldwork were examined under a Wild M5 stereomicroscope 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 was not photographed swimming alongside other members of its matrilineal group during repeated encounters over the course of the summer field season it was considered missing. If it was again missing during the repeated encounters in the following field season it was considered dead (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

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 that time. 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 each 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 or that were identified as stranded animals were considered dead.

Results

Over the course of the work initiated in 1995 and reported on here we spent a total of 816 days on the water covering 71,930 kilometers in 5,616 hours. Killer whales were encountered on 816 occasions and followed for a total of 1772 hours over 8,945 miles (Tables 1,2 , Figures 1,2)

Table 1. Summary of effort by vessels 1995-2002.

Year	*# Vessel days*	*Distance (km)*	*Time (hours)*
1995	125	12,861	884
1996	92	7,700	591
1997	126	10,597	1,099
1998	98	8,395	870
1999	98	8,359	550
2000	83	7,409	492
2001	87	7,930	493
2002	107	8,679	637
TOTAL	***816***	***71,930***	***5,616***

Figure 1. Tracklines for vessels 1995-2002

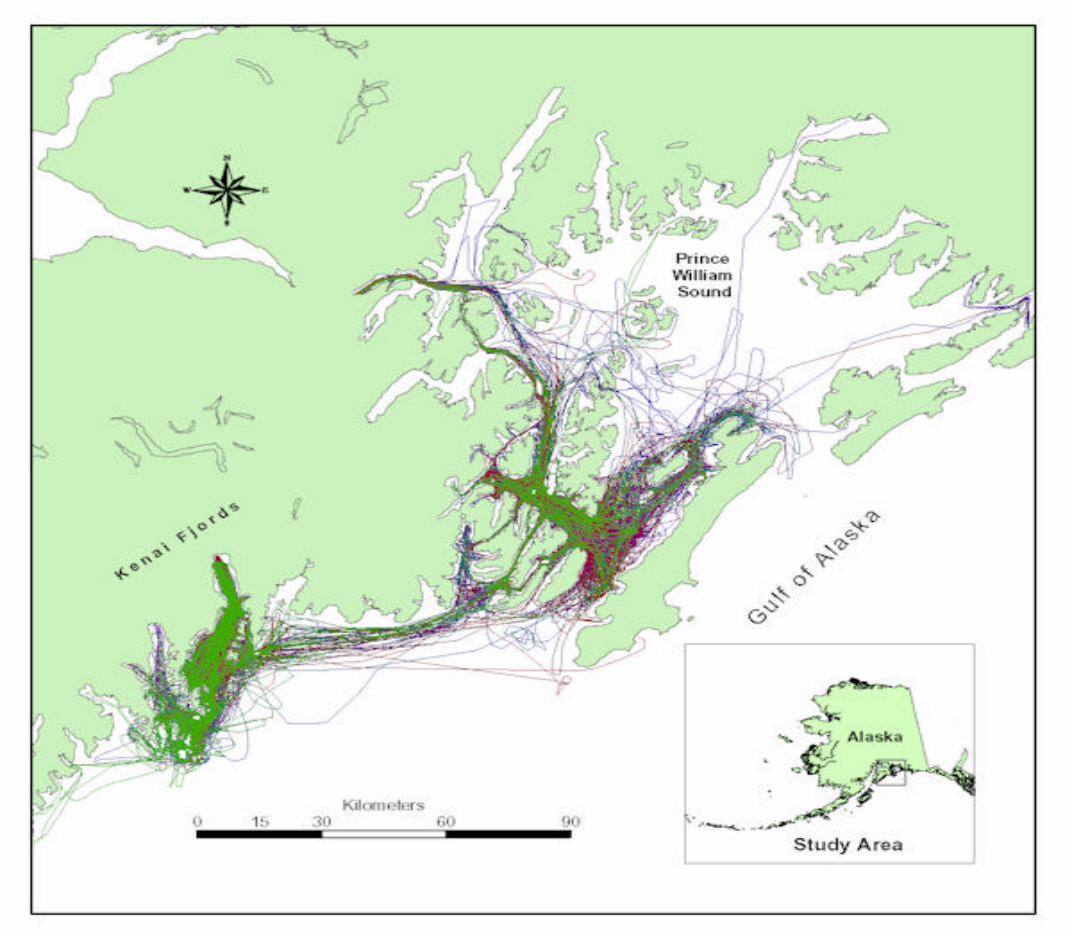
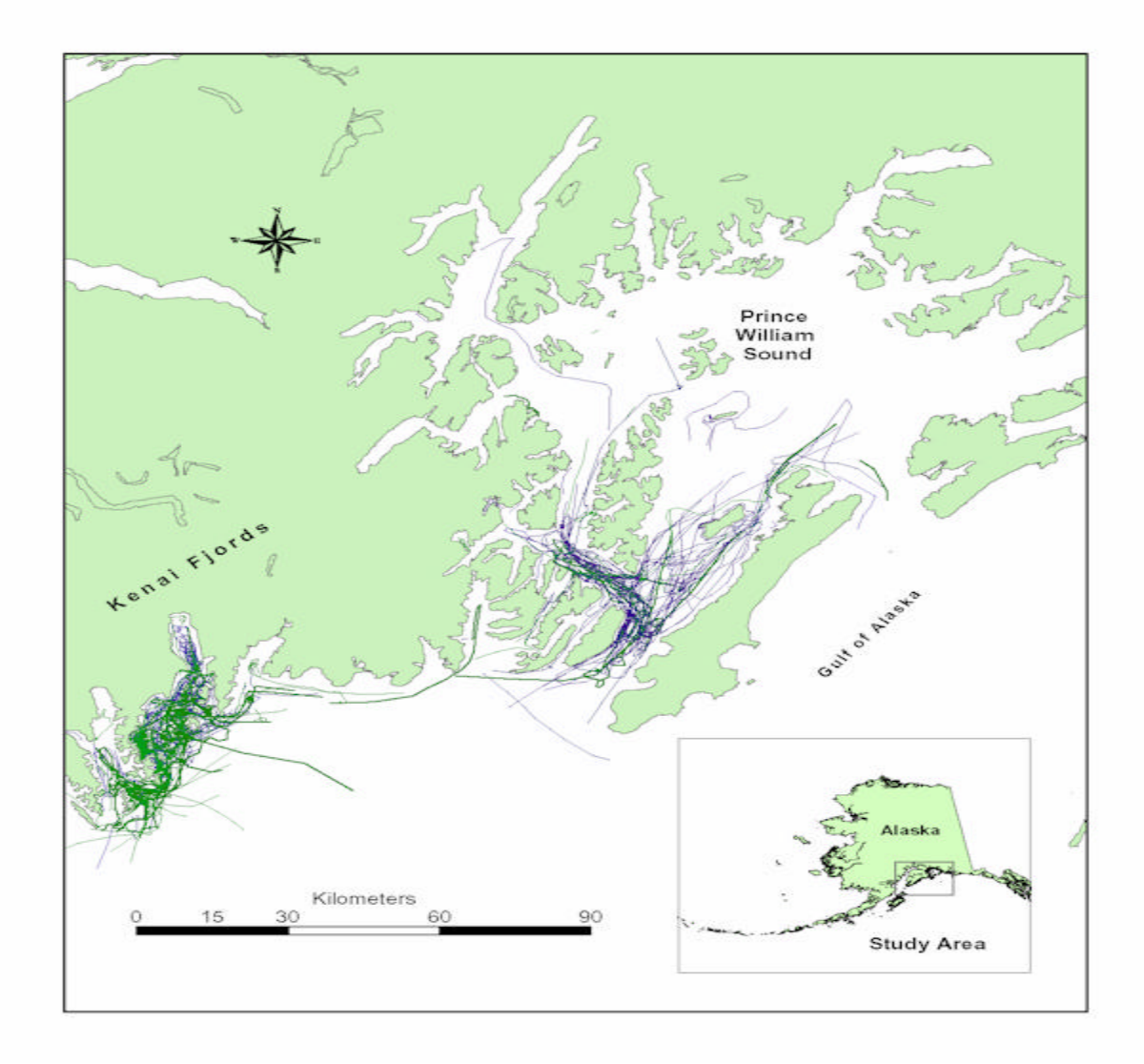


Table 2. Summary of effort for killer whale encounters 1995-2002.

Year	*# Encounters*	*Time with whales*	*Distance with whales (km)*
1995	63	234	1601
1996	32	118	922
1997	50	205	1366
1998	48	158	1127
1999	50	113	807
2000	44	142	855
2001	59	158	937
2002	66	233	1330

Figure 2. Tracklines of killer whale encounters 1995-2002.



In 2002 the 34' diesel powered *R/V Natoa* completed a total of 83 survey days in the Kenai Fjords/Prince William Sound region. The 26' high-speed motor sailer *Whale 1* completed 21 survey days in Prince William Sound, with the primary objective of humpback whale photo-identification. The 42' diesel powered high-speed charter vessel *Misty/Mariah* completed 3 survey days, Effort was divided between the Kenai Fjords and Prince William Sound areas in 2002 as it has been in all years since 1995.

In 2002 researchers were on the water a total of 107 days (40 in Prince William Sound including *Whale 1* time) and traveled a distance of 9679 km searching for and traveling with whales. Killer Whale encounter tracklines followed the same pattern observed in other years

Table 3. Effort by vessels in 2002.

Vessel	# Days	Distance(km)
Natoa	83	7791
Whale 1	21	1547

Misty/Mariah	3	341
Total	***107***	***9679***

Killer whales were encountered on 66 occasions in 2002 and researchers traveled 1329 km with killer whales (Table 4).

Table 4. Encounters with killer whales by vessels in 2002.

Vessel	# Encounters	Distance (km)
Natoa	57	1182
Whale 1	5	51
Mariah/Misty	4	96
Total	***66***	***1329***

In 2002 there were 57 encounters with resident killer whales. There were only two encounters with members of the AT1 transient group, and six encounters with Gulf of Alaska transients. There was one encounter with killer whales of the "offshore" population (Table 5, page 14).

Resident whales were encountered during all months but rates were highest in May and August (Table 5). Unfortunately AB pod was not completely photographed because of incomplete photographic coverage of the AB25 subpod which frequently travels with AJ pod and only infrequently travels with the rest of AB pod.

Encounters with transient whales were rare and scattered throughout the season although most encounters were in May when the "Kodiak Killers" (a group of five GOA transients) were encountered repeatedly and we also observed AT109, AT125 and a new calf. We are disturbed by the small number of sightings of Gulf of Alaska transients, but what was of greater concern was that we had only one encounter with AT1 transients (with AT14 in Icy Bay, Prince William Sound) which was supplemented with one encounter with AT2, 3, and 4 by the Alaska Sea Life Center that is not recorded data presented here. Only 4 whales from the AT1 group were observed this year, the lowest sighting rate for the AT1s we have ever recorded.

Table 5. Summary of Encounters 2002**.**

ENC #	REGION	DAY/MO/YR	BEGIN LOCATION	END LOCATION	POPULATION	FIELD ID	Est. # whales
1	KF	12-Jan-02	~4.5m S of harbor		Resident		20-25
1	KF	5-Mar-02	NE corner Rugged Island	3m E of Driftwood Bay	Resident		80
1	KF	7-Mar-02	1nm ESE of Caines Head	S end Cheval Narrows	Resident		80
1	KF	10-Mar-02	2m E of N end of Rugged Is	3.5m W of Rugged Island	Resident		25
1	KF	9-Apr-02	1.5m SW Callisto Head	1.5m W of S end of Rugged Is	Resident		10
1	KF	20-Apr-02	Rugged Island	S Rugged Island	Resident		40
1	KF	26-Apr-02			Resident		6
1	KF	27-Apr-02	Callisto Head	Callisto Head	Resident		9
1	KF	2-May-02	.5m N Cheval Island	off Porcupine Cove	Resident	AD5	10
1	KF	3-May-02	.5m N Callisto	3m S Porcupine	Resident	AD5 (AD5sub)	6
1	KF	4-May-02	Verdant Cove	S point Granite Island	GOA trans	GOA Trans	5
1	KF	6-May-02	Agnes Cove	Agnes Cove	Resident		6
2	KF	6-May-02	No Name Island		GOA trans		5
1	KF	11-May-02			Resident		15
1	KF	16-May-02	Thumb Bay	1m S Caines Head	Resident	AK	12
1	KF	17-May-02	off Bear Glacier	off Bear Glacier	Resident	AK	12
2	KF	17-May-02	1m N Agnes	1m N Agnes	Resident	AD5	15
1	KF	18-May-02	Chat Cove	Pony Cove	GOA trans	GOA Trans	5
2	KF	18-May-02	N end Cheval	N end Cheval	Resident	AD5	15
1	KF	19-May-02	S end Pete's Pass	Cliff Bay	GOA trans	GOA Trans	5
2	KF	19-May-02	Agnes Cove	Agnes Cove	Resident	AK	
1	KF	20-May-02	b/t Beehive and Matushka	S end Matushka	GOA trans	GOA Trans	5
1	KF	29-May-02	.5m W Cape Resurrection	.5m W Barwell Island	GOA trans	GOA Trans	3
2	KF	29-May-02	1.5m NE Pilot Rock	1.5m NE Cheval Island	Resident	AS?	30
1	KF	2-Jun-02	Agnes Bay	b/t Pony Point and Pilot Rock	Resident	AD5, AX	34
1	KF	3-Jun-02	Pony Point	off cape Aialik	Resident	AD5	15
2	KF	3-Jun-02	N end Natoa	3m SW Seal Rock	Resident	AX, ?	40-50
1	KF	4-Jun-02	N end Pony Cove	.5m NE Agnes Cove	Resident	AD5	15
1	KF	5-Jun-02	Agnes Cove	off Granite Island	Resident	AD5	15
1	KF	6-Jun-02	E of Cape Aialik	S of Agnes Cove	Resident	AD5	15
1	KF	11-Jun-02	Pony Cove	Cheval Narrows/Agens Bay	Resident	AD5	9
1	KF	12-Jun-02	.5m W Chat Island	1m E No Name Island	Resident	AD5, AK	14
1	KF	13-Jun-02	.5m SW Rugged Island	.5 W Rugged Island	Resident	AD5	9
1	KF	20-Jun-02	N end Cheval Narrows	.5m No Name Island	Resident	AD5	5
1	KF	1-Jul-02	1.5m S Chat Island	off Thunder Bay	Offshore	Offshores	45-50
1	KF	26-Jul-02	2m NE Hogan Bay	1m E Rocky Bay	Resident	AJ, AE,?	50-60
1	KF	30-Jul-02	2m SE of S tip Green Island	2m NE of N end Latouche	Resident	AB, ?	25
1	KF	2-Aug-02	Outside Barwell Island	4m NE Cape Resurrection	Resident	AB	8
1	KF	3-Aug-02	N end Harbor Island	S end of Dora Island	Resident	AN10	23
1	KF	4-Aug-02	3m S Callisto	same	Resident	AD5	7
1	KF	8-Aug-02	E side Cheval Island	S side Chat Island	Resident	AK, AN10, AI, ?	50
1	KF	9-Aug-02	1m W Chat Island	1m N Harbor Island	Resident	AN10, AK, AJ	45
1	KF	11-Aug-02	Aialik morraine	3m S Aialik morraine	Resident	AD5	14
1	KF	12-Aug-02	NE corner Natoa	N end Natoa	Resident	AD5, AN10, ?	50
1	PWS	15-Aug-02	Needle	1m NE Needle	GOA trans	GOA Trans	3
1	PWS	16-Aug-02	mid Prince of Wales Passag	b/t Needle and Point Grace	Resident	AB, AE	26
1	PWS	19-Aug-02	b/t Pleides and Squire	off Prince of Wales Passage	Resident	AB	17
1	PWS	20-Aug-02	.5m SE Needle	1m NE Channel Island	Resident	AE	20
1	PWS	25-Aug-02	1m E of N end og Green Isla	E off Rocky Bay	Resident	AB (part)	8
2	PWS	25-Aug-02	1m N Shelter Bay	2m E Sleepy Bay	Resident	AJ	32
1	PWS	26-Aug-02	1.5m W Needle	2m NE Snug Harbor	Resident	AE	19
1	PWS	27-Aug-02	2m front of Chenega Glacier	same	AT1	AT1	1
2	PWS	27-Aug-02	Chenega Point	1m N Chenega Point	Resident	AJ	3
1	PWS	28-Aug-02	.5m W Mummy Island	.25m W Mummy Island	Resident	AK (partial)	7
2	PWS	28-Aug-02	Point Helen	Fleming Island	Resident	AI, AK6, AJ (par	35
1	PWS	29-Aug-02	Fox Farm PWS	Lone Tree Light	Resident	AK6	6
1	KF	13-May-02	Spire Cove	mouth Agnes Bay	Resident	AD5	15
1	KF	14-May-02	Point N of Agnes	.25m S of Point N of Agnes	Resident	AK	12
2	KF	14-May-02	b/t Agnes and Mary's Bay	off Porcupine	Resident	AD5	15
1	KF	15-May-02	N end Cheval	off Agnes	Resident	AD5	
1	PWS	15-Jun-02	around Pleides		Resident	?	12
1	PWS	12-Jul-02	.5m out of Thumb Bay	mid Knight Island Passage	Resident	?	8
1	PWS	15-Jul-02	S end Prince of Wales Pass		Resident	?	5
1	PWS	25-Jul-02	.5m N of Pleides	mid Lower Knight Island Passa	Resident	?	20
1	PWS	26-Jul-02	Outside Fox Farm	Lower Prince of Wales Passag	Resident	?	10

Resident pods

The total number of whales in the 7 well-known resident pods in that use the Kenai Fjords/Prince William Sound region and that we have monitored since 1984 increased from 81 to 125 whales from 1988 through 2002, while AB pod declined from 36 whales to 26 whales in that same time period (Figure 3). All well known resident pods have increased or are at the same numbers as in 1984 except AB pod (Figure 4). Three resident pods, (AG, AF05, and AF22) that apparently center their range in southeastern Alaska also increased in number during this period. They totaled 47 whales in 1988 and 85 whales in 2001 and were not photographed in 2002.

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. There was one recruited calf and no mortalities in AB pod in 2000. In 2001 we observed two new calves and one new mortality, all in the AB25 subpod which travels with AJ pod. The whale AB57 was a new calf to AB33 (2001) and AB58 was a new calf to female AB25. The mortality, AB51 (born in 1996) was the juvenile offspring of AB25. The total number of whales in AB pod is now estimated at 26 although the pod was not completely photographed in 2002.

Members of AB pod were encountered on only 7 occasions in 2002. The entire pod (both the AB17 and AB25 subpods) was not encountered together and the AB25 subpod (which still often travels with AJ pod) was never completely photographed. The first encounter with the pod was on 5 March, and they were last photographed on 25 August. One new calf recruited to the AB17 subpod in 2002 (AB59 born to AB26) and there were no mortalities. However due to poor photographic coverage of the AB25 subpod (AB51, 57, and 58 were not photographed) we cannot determine the the total number of whales for AB pod in 2002 and must use the figure of 26 whales from the 2001 census as our best estimate. As has been the case in recent years, AB pod was not present during most of the summer field season (May, June and July) although they were present in Resurrection Bay in late winter as determined by the remote hydrophone recordings as well as field observations.

A total of 8 new calves were recruited into the well-known resident pods other than AB pod in 2002 (Table 6), and these pods are maintaining a growth rate averaging about 3.3% per year. These new calves AJ 48, calf of AJ4; AJ49 calf of AJ20; AK18 calf of AK7, AK19 calf of AK10; AN61 calf of AN8; AN62 calf of AN45; and AE25, calf of AE2 and AD36 calf of AD21. There were no new mortalities in these pods. AE pod also had a previously unrecorded year old calf, AE24 calf of AE17 that was not recorded in 2001 because of incomplete photographic coverage of the pod in that year.

Mortalities observed in 2001 were confirmed in 2002 for all pods. Births and deaths are listed by pod for 1984-2001 in Table 6 and annual mortality and recruitment rates are listed in Table 7. However, one animal presumed dead returned to its pod in what was an extremely unusual event. AD4, an older, post reproductive female that had lost all her offspring and wandered between AD05 subgroups in previous years, had not been photographed since 1999, but returned to the pod in 2002. This is the first time an animal missing for years has returned and is no doubt linked to her unusual position as a post reproductive female with no surviving offspring. She is the last in her matriline. The lack of strong social ties may have induced the tendency to wander.

Figure 3. The number of resident killer whales in AB pod, in seven other Prince William Sound/Kenai Fjords resident pods and in three Southeastern Alaska resident pods 1984-2002.

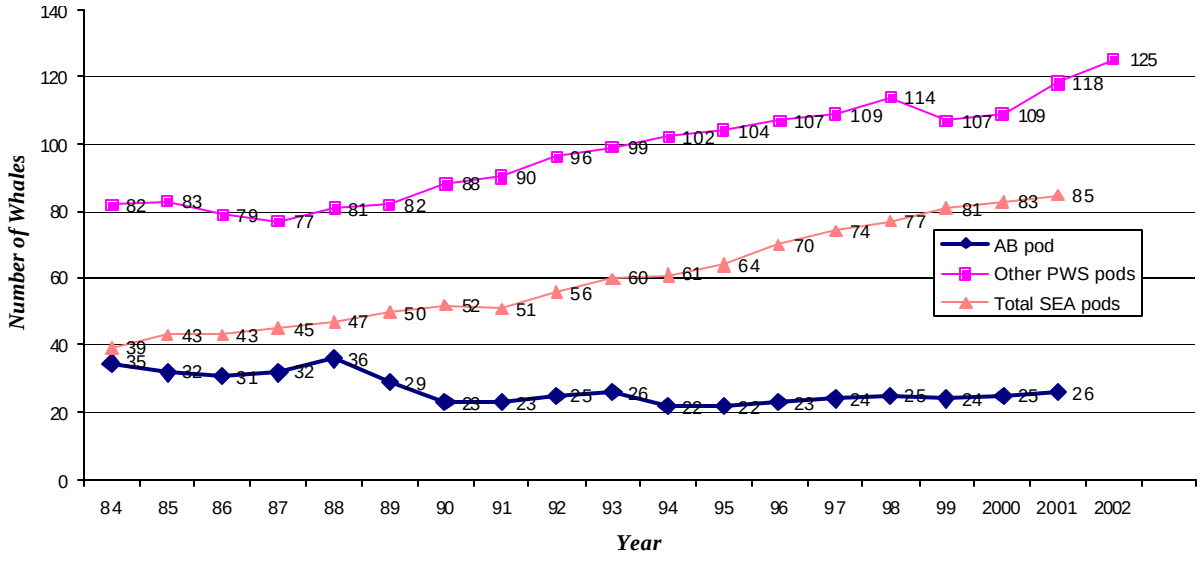


Figure 4. Number of whales in AB pod and in seven other major resident pods 1984-2002.

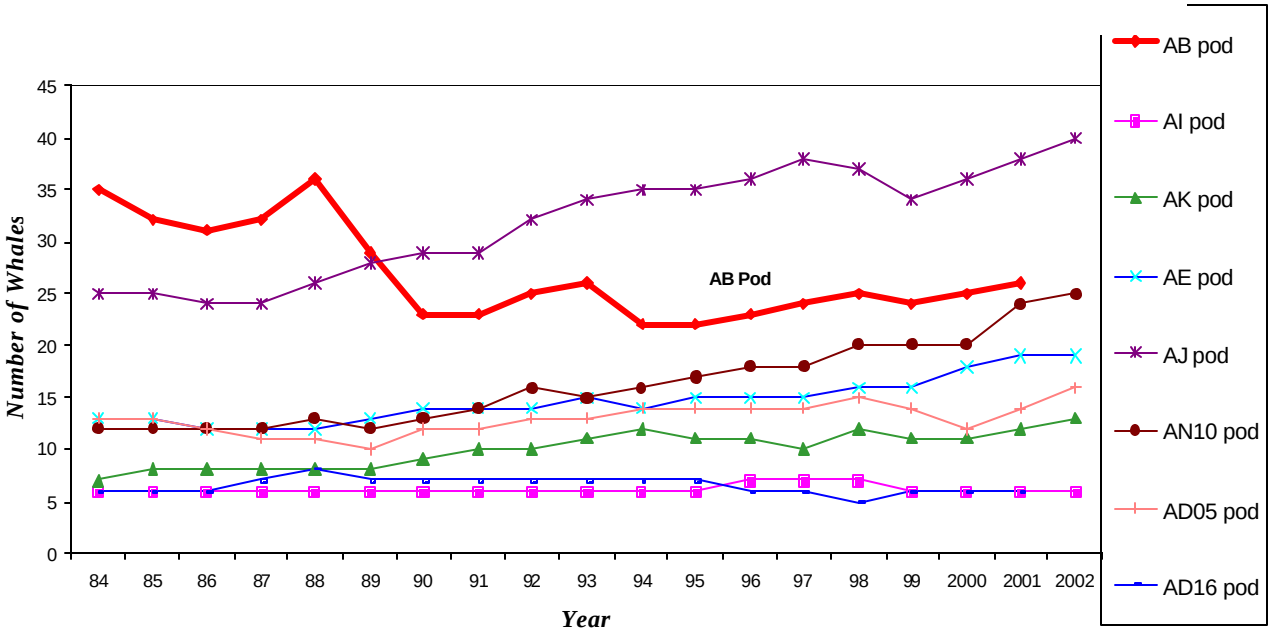


Table 6. Recruitment and mortalities in Prince William Sound and Southeast Alaska resident pods in 2002.

	Recruitment in Prince William Sound/Kenai Fjords Resident Pods [whale#(mothers#)]									***Southeast Alaska Resident Pods***		
POD	***AB***	***AI***	***AK***	***AE***	***AJ***	***AN10***	***AD05***	***AD16***		***AF05***	***AF22***	***AG***
YEAR ***85***			8(6)	13(11)							24(6)21(8)	18(8)19(11)
86	***36(23),37(6)***		9(2)									
87	***38(31),39(25)***					38(10)				31(20)29(15)		
88	***40(14), 41(8) 42(32)***			15(10)	26(22)27(20)	40(35)		20(16)		33(11)	28(4)	16(11)
89	***43(17), 44(22)***			16(2)17(5)	28(24)29(8)		26(11)			36(5)	27(7)26(8)	17(5)
90			10(2)	18(11)	30(3)	41(8)	21(5)24(7)			35(20)	30(10)	20(4)21(10)
91	***45(16)***		11(6)			45(35)				38(11)		
92	***46(25),47(32)***				31(24)32(22)33(13	46(10)47(11)	22(7)			34(15)	44(6)40(22)	22(5)23(15)
93	***48(26)***		12(7)	19(11)	34(3)35(8)36(4)					37(5)51(20)55(11)42(13	48(4)	24(11)
94	***49(22)***		13(2)		37(18),38(20)	48(8)	23(8)				39(16)	25(8)
95				20(2)		49(11)	25(5)	28(16)		43(11)41(25)		26(6)
96	***50(26),51(25)***	7(4)			39(13)	50(35)51(12)	27(11)			54(20)49(23)	45(6)46(10)47(8	27(15)28(5)
97	***52(33), 53(27)***				40(3)41(4)	54(10)		29(18)		50(11)		29(4)30(11)31(10
98	***54(17)***	8(3)	14(7)15(9)	21(5)		55(8)56(11)	30(7)			52(12)		
99	***55(39)***				42(24)43(22)		31(8)	32(16)		53(13)56(20)57(15)	64(10)	33(6)
2000	***56(22)***		16(2)	22(10) 23(11	44(13)45(3)			33(20)		58(25)60(11)59(23)	65(4)	32(19)
2001	***57(33),58(25)***		17(06)	24(17)	46(27),47(28)	57(10)58(12)59(35)60(41	34(11)35(8)			61(17)	63(16)67(8)	34(15
2002	***59(26) *****		18(7)19(10)	25(2)	48(4)49(20)	61(8)62(45)	36(21)	**				
	** not completely photographed											
	Mortalities in Prince William Sound/Kenai Fjords Resident Pods [by whale number]									***Southeast Alaska Resident Pods***		
POD	***AB***	***AI***	***AK***	***AE***	***AJ***	***AN10***	***AD05***	***AD16***		***AF05***	***AF22***	***AG***
YEAR ***85***	***9,15,34***			8-								
86	***1,7,12***		5-	4-	23-		9-	17-				
87	***28-***					6-	1-	15-				
88	***6-***			7-								1-
89	***13,18,21,23,30,31,37***			12-		2-	3,10				2-	
90	***8,19,20,36,42,44***										1-	9-
91	***29-***										3,7	
92												
93					5-	5-						7,16
94	***2,16,38,41,48***			13-	11-					55-		
95			4-				23-					
96	***4-***					1-	26-			43-		
97	***3-***		11-			49-						
98		8-			6-							
99	***5,52***	1-	3-		9,12,16,17,18		12	18,29		36-		
2000			8-				7,30	14-		38,53,54		
2001	***51-***										39*,48*	
****2002***	********		13-	9-		3-		**		**	**	**
	*to be confirmed in2003		** not completely photographed									
[#84/#01]	[35/26]**	[6/6]	[7/13]	[13/19]	[25/40]	[12/25]	[13,16]	[6/6]**		[12/30]	[12/25]	[15/30]

Table 7. Recruitment and Mortality rates by year for resident pods in PWS/KF and Southeast Alaska 984-2002.

	Recruitment rates in PWS/KF Resident Pods										***Southeast Alaska Resident Pods***			
	AB	***AI***	***AK***	***AE***	***AJ***	***AN10***	***AD05***	***AD16***	***non-AB total***		***AF05***	***AF22***	***AG***	***Total SEA***
85	***0***	0	14.3	7.7	0	0	0	0	2.4		0	16.7	13.3	7.7
86	***6.3***	0	12.5	0	0	0	0	0	1.2		0	0	0	0
87	***6.4***	0	0	0	0	8.3	0	0	1.3		16.7	0	0	4.7
88	***15.6***	0	0	8.3	8.3	8.3	0	25	6.5		7.1	7.1	5.9	6.7
89	***0***	0	0	15.4	7.7	0	9.1	0	6.2		6.7	13.3	5.9	8.5
90	***0***	0	12.5	7.7	3.6	8.3	20	0	7.3		6.3	6.3	11.1	8
91	***4.3***	0	11.1	0	0	7.7	0	0	2.3		5.9	0	0	1.9
92	***8.7***	0	0	0	10.3	14.3	8.3	0	6.7		5.6	14.3	10.5	9.8
93	***4***	0	10	7.1	9.4	0	0	0	5.2		21.1	6.3	4.8	10.7
94	***3.8***	0	9.1	0	5.9	6.7	7.7	0	5.1		0	5.9	5	3.3
95	***0***	0	0	7.1	0	6.3	7.1	20	3.9		9.1	0	4.8	4.9
96	***9.1***	16.7	0	0	2.9	11.8	7.1	0	4.8		8.7	16.7	9.1	10.9
97	***8.6***	0	0	0	5.4	5.5	0	16.7	3.7		4	0	12.5	5.7
98	***4.2***	14.3	20	6.7	0	11.1	7.1	0	6.4		3.8	0	0	1.4
99	***4***	0	0	0	5.4	0	6.7	14.3	3.5		11.1	4.3	3.7	6.5
2000	***4.2***	0	0	12.5	5.9	0	0	16.7	5.6		10.3	4.1	3.6	6.2
2001	***8***	0	9.1	5.5	5.5	20	15.4	0	10.3		3.4	8	3.4	4.8
2002	*	0	16.6	5.3	5.3	8.3	6.6	*	6.8		*	*	*	*
	Mortality rates in PWS/KF Resident Pods										***Southeast Alaska Resident Pods***			
	AB	***AI***	***AK***	***AE***	***AJ***	***AN10***	***AD05***	***AD16***	***non AB total***		***AF05***	***AF22***	***AG***	***Total SEA***
85	***8.6***	0	0	7.7	0	0	0	0	1.2		0	0	0	0
86	***9.4***	0	12.5	7.7	4	0	8.8	16.7	6		0	0	0	0
87	***3.2***	0	0	0	0	8.3	8.3	20	3.8		0	0	0	0
88	***3.18***	0	0	8.3	0	0	0	0	1.3		0	0	5.9	2.2
89	***19.4***	0	0	8.3	0	7.7	18.2	0	4.9		0	6.7	0	2.1
90	***20.7***	0	0	0	0	0	0	0	0		0	6.3	5.6	4
91	***4.31***	0	0	0	0	0	0	0	0		0	11.8	0	3.8
92	***0***	0	0	0	0	0	0	0	0		0	0	0	0
93	***0***	0	0	0	3.1	6.3	0	0	2.1		0	0	9.5	3.6
94	***19.2***	0	0	6.7	2.9	0	0	0	2		4.3	0	0	1.7
95	***0***	0	8.3	0	0	0	7.1	0	2		0	0	0	0
96	***4.5***	0	0	0	0	5.9	7.1	0	1.9		4.3	0	0	1.6
97	***4.3***	0	9	0	0	5.5	0	0	1.9		0	0	0	0
98	***0***	14.3	0	0	2.6	0	0	0	1.8		0	0	0	0
99	***8***	14.3	8.3	0	13.5	0	13.4	28.6	9.8		3.7	0	0	1.3
2000	***0***	0	9.1	0	0	0	13.3	16.7	3.7		10.3	0	0	3.7
2001	***4***	0	0	0	0	0	0	0	0		0	8	0	2.4
2002	*	0	8.2	5.3	0	4.2	0	*	2.5		*	*	*	*
# in pod84/01	***[35/26]***	[6/6]	[7/13]	[13/19]	[25/40]	[12/25]	[13/16]	[6/6]	***[82/125]***		[12/30]	[12/25]	[15/30]	***[39/84***
	****entire pod not photographed***													

We encountered members of 15 different resident pods in 2002 (Table 8), a total of 241 individuals. Pods that were completely photographed in 2002 included AD05, AE, AJ, AI, AK, AN10, AH, AH20,AS, AS30, AY, AX30, AX40 . Also, three of the four matrilines that compose AX pod (see 1999 catalogue, Matkin et. al. 1999) were photographed in addition to two new resident pods, AH1 and AH20. AS pod has now been split into two pods, AS and AS30 pods.

Table 8. Resident pods: number of whales in 2002.

Pod	#Whales
AB*	26
AJ	40
AN10	25
AI	6
AE	19
AK	12
AD16*	6
AD5	16
AX30**	12
AX40**	13
AY	13
AS30**	12
AS**	21
AH**	8
AH20**	12
TOTAL	***241***

* pod not completely photographed

** AX, AH, and AS pods have been split to reflect changes in association patterns between matrilines in these pods.

Transient whales

Only 4 of the original 22 whales from the genetically unique AT1 group were photographed during 2 encounters in 2002. Whales photographed included AT2, AT3, AT4 and AT14. In addition, the whale stranded in 2001 on Hinchinbrook Island was another AT1 whale (determined by mtDNA) and was likely AT10 who has been missing for several years.

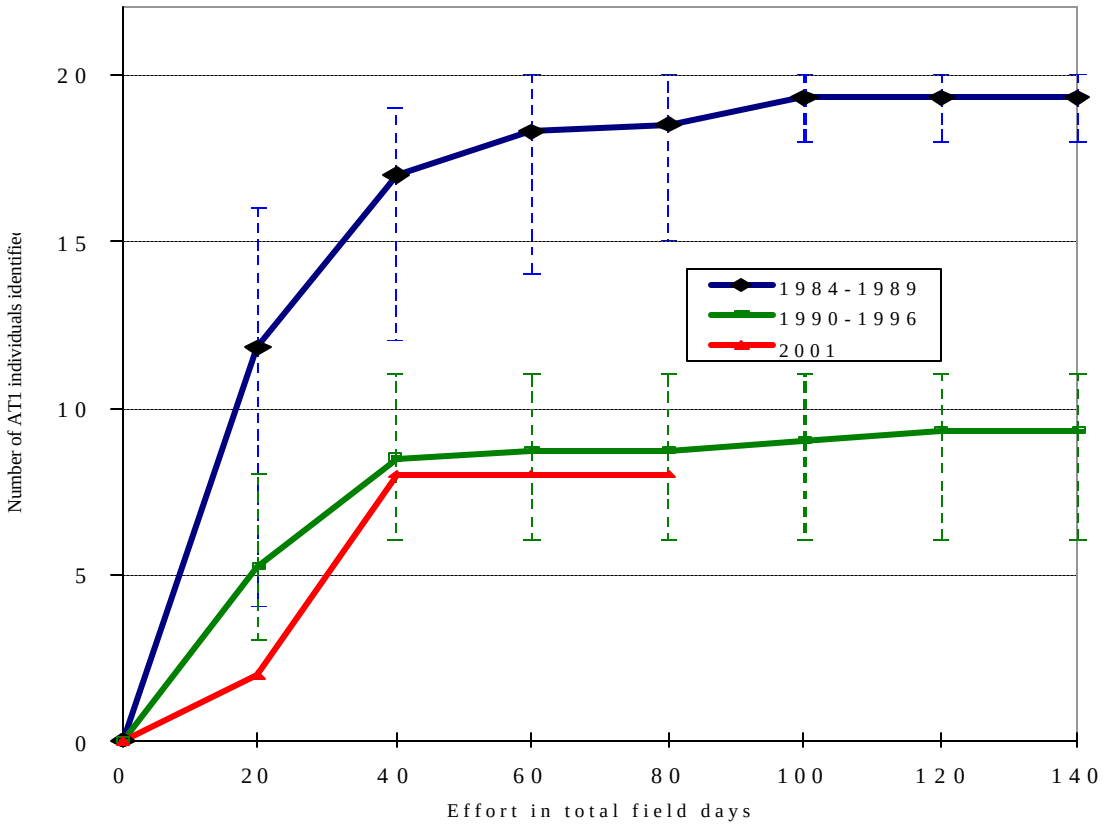
Thirteen whales in the AT1 group have been missing for ten years or more and are considered dead or have been found dead and stranded. The group numbers only 9 individuals as of late 2002. Since 1989, the number of AT1 individuals identified annually has been 12 or less despite a field effort that exceeded 200 vessel days in 1990 and totaled 120 days in 1997, 98 days in 1998, 83 days in 2000, 87 days in 2001 and 107 days in 2002. The sighting rate in 2002 was the poorest recorded, and

hence we use sighting rates from 2001 in Figure 5. There were no new calves identified in the AT1 group in 2002, 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 2001 the individuals sighted per effort was slightly below the average for the 1990-1997 period.

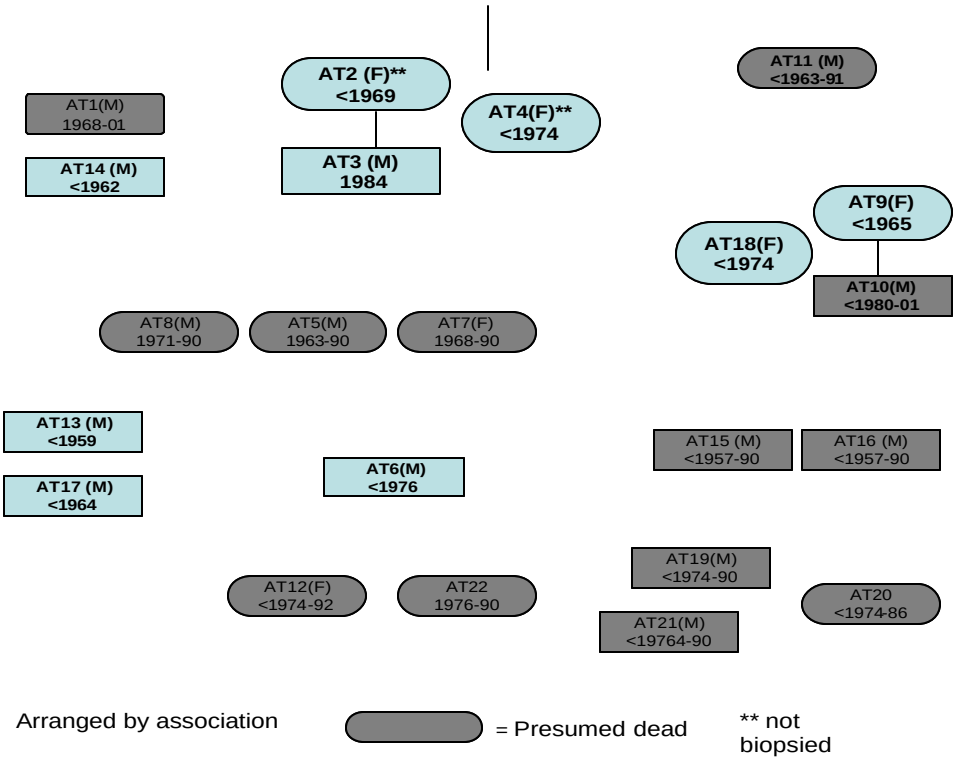
Both before and after 1989, all of the AT1 whales photographed in a particular year were generally seen in the first 20 to 60 days of the field season, however this was not the case in 2002, when AT1 whales were not sighted until late in the season.

Figure 5. Average number of AT1 transient whales seen in years with effort greater than 60 field days and actual number of whales identified in 2001. (error bars = range)



The social structure of the AT1 transient group is more fluid than in resident pods. The entire group has only been observed together on one occasion and associations of more than 5 AT1 whales are rare. None of these whales have ever been observed in association with killer whales other than members of their own population. In the diagram below the basic subgroupings within the population are diagrammed. There are only 4 females (AT2, AT4, AT9, and AT18) remaining that might potentially recruit a calf and two of those whales (AT2 and AT9) are possibly post reproductive with uncertain dates of birth. Currently the AT1 population is being considered for listing under the Marine Mammal Protection Act as a depleted population.

Figure 6. Diagramatic representation of the AT1 transient population as it appeared in 1988 prior to the *Exxon Valdez* oil spill. Individuals are grouped by their associations, animals in dark grey are missing and presumed dead. Males are in square boxes.



Eight non-AT1 transients made appearances in the study area. These included AT109 and AT111 with a new calf. These whales traveled as a trio and were observed sporadically from May to August. The 5 transient whales nicknamed the "Kodiak Killers" (which include AT51 and are receiving new numbers) were observed only six occasions in May. On two occasions they were observed preying upon juvenile Steller sea lions.

Discussion

In 2002, the final year of the study reported on here, we were unable to assess changes in AB pod since the previous year due to incomplete photographic coverage of the AB25 subpod, the group most often associated with AJ pod since the *Exxon Valdez* oil spill. One new calf was recruited; however, it is unclear whether there were mortalities in the AB25 subpod. The AB25 subpod still appears to spend most of its time in the company of AJ pod, although it maintains the AB pod vocal dialect. There were no new mortalities in change AB17 subpod and one new calf recruited to AB26.

There were no changes in the small AB10 subpod. The current number of 26 whales in AB pod is far from the prespill level of 36 whales; however, a slow recovery appears to be underway.

Recent population modeling indicates that recovery can not be expected for some time, probably not for another decade or more. Using a conservative intrinsic rate of increase of 2.4%, we would not predict recovery to the 1988 level of 36 whales until 2015. This is over twice as long a recovery time as we estimated immediately following the spill. Although there were additional mortalities due to changes in social structure following the spill (i.e. death of orphaned calves), the extended recovery time is primarily due to the very atypical loss of reproductive females and juveniles at the time of the spill. As indicated by our population modeling (see following section on Population Modeling), AB pod would have recovered by 2001 had it not been for the loss of reproductive females and juveniles at the time of the spill. Our modeling also suggests that conditions in the northern Gulf of Alaska (including southeastern Alaska) have been near optimal for resident killer whales during the past decade as evidenced by the rate of increase in Prince William Sound/ Kenai Fjords pods, and the continued steady growth of the population in 2002. If conditions were not optimal, the recovery of AB pod would likely be extended beyond our current projection. This underscores the difficulty resident killer whale pods have recovering from anthropogenic or natural disasters, particularly those that involve loss of reproductive females and/or juvenile females, even during periods that are optimal for population growth.

AB pod maintained its basic pattern of absence from the study area during the spring and summer months (May thru early July) with sporadic appearances that begin at the end of July and may continue through the fall. They appear to spend far less time in the study area during the spring summer and fall than prior to the spill. AB pod may be more consistently present in the late winter period (February-March) in Resurrection Bay when their calls (mixed with AJ pod calls) are repeatedly recorded from the remote hydrophone. Since in most instances we have only recordings, it is not clear whether the entire pod is present or it is only the AB25 subpod that often travels with AJ pod.

The loss of another individual (apparently AT10) in the AT1 transient group in 2001 was confirmed by genetic analysis of skin from the beached carcass. This sets the number of AT1 whales remaining at 9 individuals, compared to a total of 22 prior to the 1989 spill. Again, there has been no observed recruitment into the AT1 group in 2002 and recruitment has not been observed since 1984. It is uncertain if any of the AT1 whales are capable of recruiting a calf since there has been no recruitment in 17 years. High contaminant levels (PCBs and DDTs) found in blubber biopsies from members of this group suggest that contaminants could play a part in the low recruitment rates.

The surviving members of the AT1 group are seen less frequently than in pre-oil spill years. Several factors may be responsible. 1) There are far fewer AT1s than in years past. 2) They may be forced to range more widely in search of prey because of the severe reduction in harbor seal numbers in the region. 3) They may be forced to forage further offshore for porpoises, reducing our ability to locate them. Although we no longer observe and photograph all of the remaining nine whales in a given year, they have not been observed or photographed in adjacent areas (northern Southeastern Alaska and Kodiak) despite recently expanded efforts in those regions. It is unlikely that these whales range far from the Prince William Sound/Kenai Fjords. 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 review).

Despite the fact that the AT1 group continues a slow decline, the steep decline at the time of the oil spill (loss of nine of 22 individuals in 1989 and 1990) is unlikely an event that was simply coincidental considering: 1) The lack of mortalities in this group in the five years they were studied and

enumerated prior to the spill. 2) The presence of several of the missing whales in the slick alongside *the Exxon Valdez* at the time of the spill. 3) The repeated presence of many individuals in the spill zone in 1989 and, 4) The availability of oiled harbor seals following the spill. Although other factors such as high contaminant levels and the continued decline of their harbor seal prey may be contributing to the decline and lack of recovery, the major factor in the overall decline of the AT1 group since 1988 appears to be the effects of the *Exxon Valdez* oil spill.

POPULATION DYNAMICS

Introduction

After 17 years of monitoring individual life histories (Matkin et al 1999b), establishing genealogies (Matkin et al 1999a), and using comparative data from British Columbia (Olesiuk et al 1990) we have constructed a population model for the southern Alaska resident killer whales using data collected from 1984-2001. There are limitations to the modeling approach due to the relatively short duration of the study, we have observed these whales for only 17 years, or approximately one generation. Female calves that were born at the beginning of the study are now beginning to produce calves and males born at the beginning of the study are becoming sexually mature as evidenced by the growth in their dorsal fins. However, sufficient data is not yet available to calculate certain paramenters such as average age of first reproduction for females or average age of sexual and physical maturity for males. Nonetheless, with these parameters borrowed from the British Columbia population we were able to construct a model and calculate the major population parameters. The southern Alaska population model described here was constructed exlusive of AB pod so that parameters for this pod could be compared with the overall model to assess the effects of the *Exxon Valdez* Oil Spill. The goals of this our modeling exercise were to: 1) estimate life history parameters (including survival and recruitment rates) and compare to previous estimates for British Columbia northern residents 2) incorporate life history parameters into the population model (using life tables and Leslie-type matrices) 3) use the model to estimate population parameters (life expectancy, longevity, reproductive value) 4) Illustrate the application of model with an assessment with impact of the *Exxon Valdez* oil spill on AB-Pod.

Methods

Field methods used in this part of our analysis were the same as those used for photoidentification and enumeration purposes described in the previous "Field Methods" section. However in addition to identifying each whale in the pods used in our model in each year, it was necessary to age each whale. Killer whales were aged using the following criteria:

1) Animals born during study were aged on basis of year first observed or on their size if they were not seen in the year of birth. In addition, three females born several years prior to study were

aged on the basis of size when first seen. These are referred to as known-aged animals, although the latter might actually be known to within +/- 1 or 2 years.

2) Males that were juveniles when first seen but too large to estimate based on size were aged by subtracting mean age of onset of sexual maturity (13.4 years) from the year the dorsal fin began to sprout.

3) Males that were sexually but not physically mature when first seen were aged by subtracting mean age of onset of physical maturity (18.8 years) from the year by which the dorsal fin was fully developed.

4) Males that were physically mature when first seen were aged on basis of the year they were first seen (these are considered minimum ages).

5) Animals that were approaching adult size when first seen but died before maturing were aged based on their size when first seen (these are considered crude ages).

6) Females that were juvenile-size when first seen were aged by subtracting mean age of first recruitment (14.8 years) from year they gave birth to their first viable calf.

7) Females that were adult-size when first seen were also aged by subtracting mean age of first birth (14.8 years) from year of birth of oldest known calf. Since these females may have given birth but lost older progeny prior to the start of the study, a correction was applied to account for this calf loss. For example, if the female had lost her first calf, she would have been one calving interval older; if she had lost her first two calves, she would have been two calving intervals older, etc. Based on calving rates and survival rates of calves, a probabilistic correction factor was calculated as outlined in Section 3.1.2 of Olesiuk et al. 1990. The corrections increased as a function of the age of the oldest known offspring when first seen, and ranged from 0.7 when the oldest known offspring was first seen at age 0, to 1.4 when first seen at age 10, to 2.8 when first seen at age 20, to 5.4 when first seen at age 30. (Note: for 6 females, the oldest offspring were minimum-aged males, so their ages and the correction factors are also minimums).

The age estimates are contingent upon three main parameters: 1) age at birth of first viable calf; 2) calving intervals; and 3) juvenile survival rates (the latter two are required for the correction factors

Juvenile mortality rates were based on sample of 128 known-aged animals. In our calculations males and females were combined combined and individuals placed in subcategories to track changes by age (age specific mortality rates). Categories included: 0-1 years, 2-3 years, 4-6 years, 7-10 years and 11-15 years. Mortality rates were calculated from from survival probabilities:

$$MR(x) = D(x) / N(x)$$

D(x)=Number Dying

N(x)=Total Number Observed

Female mortality data consisted all females that matured during or prior to start of study. The categories used to look at age specific rates were: 15-20 years, 20-29 years, 30-39 years, 40-49 years and 50+ years. The same formula was used as in the case of juvenile mortality calculations. For mature females

also calculated intervals between the birth of successive viable calves and estimated fecundity from the proportion of mature females giving birth each year:

$$Fec(x) = \frac{\text{Number Calving at Age(x)}}{\text{Total Number Females Age(x)}}$$

We examined reproductive senescense by calculating the proportion of females that were post-reproductive:

$$PR(x) = \text{Fec-total(x)} / \text{Fec-reproductive(x)}$$

A female was defined as post reproductive when she had not calved in 10 years or more.

Caculation of male mortality rates calculated for males that attained sexual or physical maturity during the study using formulas similar to those used to calculate juvenile survival rates. The age categories used for males were: 15-20 years, 20-24 years, and 25-34 years. The rate for those >35+ years was estimated from B.C. data (Olesiuk, pers. comm.) because of small sample size in our study.

The life history parameters were incorporated into life tables and a Lesile-type matrix population model and Lotka-Voltera equations (finite approximations) to determine population parameters and model its dynamics. The estimated annual recruitment and survival rates were calculated:

Recruitment:

$$N_{m(0)} = N_{f(0)} = \Sigma N_{f(x)} \bullet Fec_{(x)}$$

Survival:

$$N_{f(x+1)} = N_{f(x)} \bullet (1\text{-}MR_{f(x)})$$
$$N_{m(x+1)} = N_{m(x)} \bullet (1\text{-}MR_{m(x)})$$

Where N(x), Nf(x) and Nm(x) represent the number of juveniles of either sex, adult females and adult males aged x in that year, MR(x) is the age-specific mortality rates of juveniles aged less than 15 of both sex as per Table 9 in Olesiuk et al (1990) but updated to included data to the late 1990s, and MRf(x) and MRm(x) represent the age-specific mortality rates of females and males aged 15 or greater as per Table s 11 and 12 respectively in Olesiuk et al (1990) again updated for data to the late 1990s.

We compared the observed and expected number of deaths, and to dampen year-to-year fluctuations due to stochastic events (births and deaths are integers, where predicted values are real numbers), we also calculated 3-year running means of the ratio of observed to expected values.

Since there were far more deaths in AB-pod than expected in 1989-90 following EVOS, we examined the effects of these losses. In order to estimate the lost production from these animals, we projected their production in the decade following their disappearance:

$$\boldsymbol{N}t+1 = \boldsymbol{M} \bullet \boldsymbol{N}t$$

Where $\boldsymbol{N}t$ is a vector giving the number of animals by age and $\boldsymbol{M}$ the Leslie projection matrix giving the age-specific fecundity and survival rates (see Section 4.1 in Olesiuk et al 1990 for details).

For the six juveniles of unknown sex, we assumed half were female (each was counted as 0.5 females in the vector). In each year, we summed the estimated number of animals that would have been

born to the animals that disappeared and would have survived, and added them to the observed size of AB-pod.

Results

We have identified over 450 resident killer whales in the southern Alaska population during this study (Matkin et al 1999c), however, we were able to regularly locate and re-identify only the 319 of these whales that were observed in 11 pods and of these 152-229 were alive at any one time. These pods and the number of whales in each as of 2001 are listed in Table 9. It is the individuals in these pods, representing a large subset of the entire population, that were used in our analysis. The results of this analysis were considered representative of the entire population.

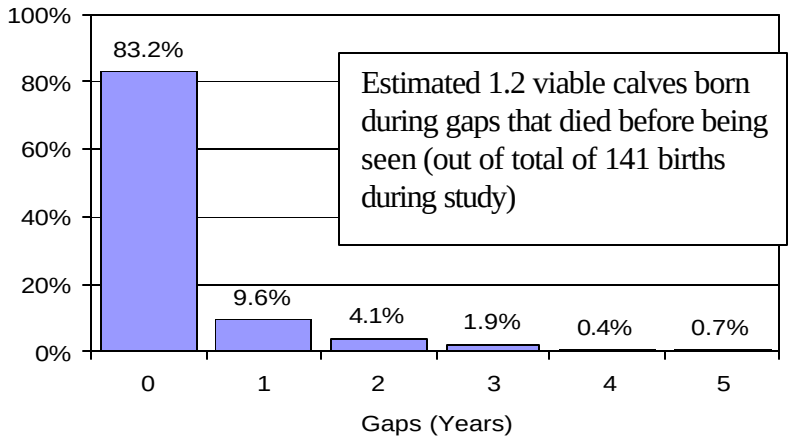
Table 9. The current number of individuals in each pod and the identification numbers for every whale tracked in those pods during the study 1984-2001.

Pod	***Individual identification numbers***	***Total 2001***
AB	AB1-58	26
AD05	AD1-12,19, 21-25,27,30-31,34-35	14
AD16	AD 13-18, 20,28-29,32-33	6
AE	AE1-22	18
AF05	AF5,11-15,17-18,20,23,25,2931-38,41-43,49-61	30
AF22	AF1-10, 16,19,21-22,	25
AG	AG1-34	30
AI	AI1-8	6
AJ	AJ1-47	38
AK	AK1-17	12
AN10	AN1-3, 5-8, 11-12,38,40-41,45-51,54-60	24

The basis of our study was the annual census of the individuals in these eleven pods, however not all individuals were photographed and identified in each year. This resulted in some gaps in our data that

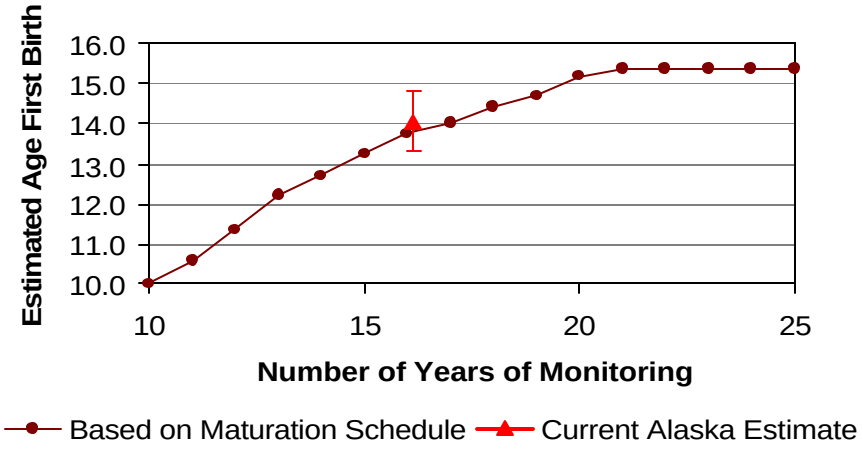
required interpolation in some instances (Figure 7). However in 83.2% of our resights there were no gaps between years, and in 9.6% there were one year gaps. There were gaps of three or more years in 7.2% of the resights.

Figure 7. Gaps in sampling of individuals during annual surveys 1984-2001.



Data were insufficient due to the short term of the study to accurately determine age of first reproduction for the southern Alaska resident killer whale population. At this time we have observed only five known-aged females give birth to their first viable calves. Since these were the first maturing females, their age at first reproduction was likely low-biased compared to the average. However if we include AB33, AD18 and AN12, which were first seen at estimated ages of three, two, and three years respectively, and gave birth at estimated ages of 16, 15 and 15 respectively, the age of first viable reproduction appeared similar to that determined for northern resident killer whales in British Columbia which was 14.75 years (Figure 8). This figure was used in calculations in our model.

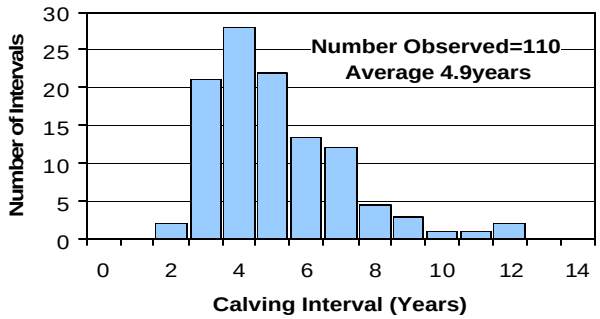
Figure 8. Average age at first reproduction for British Columbia resident killer whales by years of observation with current PWS/KF estimate.



Since it was also too soon to accurately determine the average male age of sexual maturity in our study, we used the figure of the 14.4 years determined in British Columbia (Olesiuk et al 1990). Indications are that the actual age in our area is very close to this.

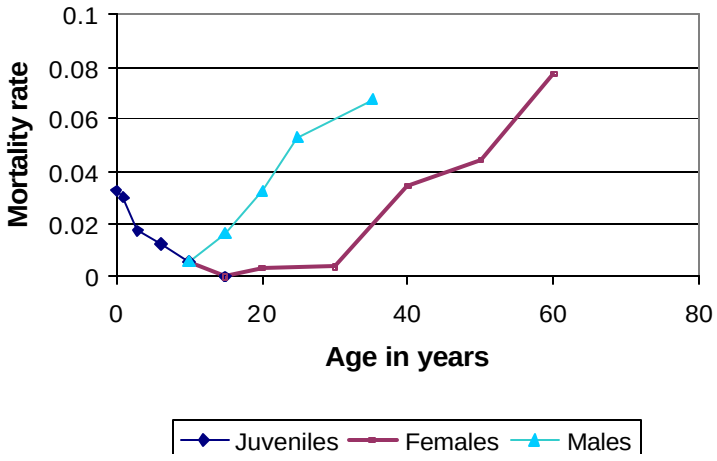
Calving intervals were determined for known reproductive females in the Alaskan population (Figure 9). Calving intervals in the northern B.C. residents and PWS/Kenai Fjords residents were very similar, (mean 5.2 and 4.9, respectively).

Figure 9. Calving intervals for Prince William Sound/ Kenai Fjords resident killer whales 1984-2001.



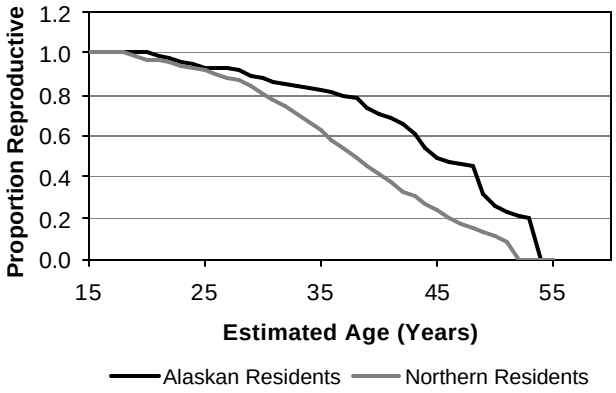
We examined survival rates for various sex and age classes for southern Alaska residents (Figure 10) The mortality rates follow the classic U shaped mammalian curve with higher mortality rates for very young whales which decrease during reproductive years and then increase with age. Survival rates for juveniles of both sexes up to age 15 (sexual maturity) were very similar for our population and the northern B.C. residents: 0.79 for all Alaskan pods except AB pod, and 0.77 in northern B.C. residents. In the Alaskan population (excluding AB pod), the adult female survival rates over the 25-year reproductive lifespan were 0.69. This is substantially lower than the British Columbia rate 0.85 (Olesiuk et al 1990). Primarily this is due to the sharp increase in mortality of females in their late 30's in the Alaskan population.

Figure 10. Age specific mortality rates for Prince William Sound/Kenai Fjords resident killer whales 1984-2001.



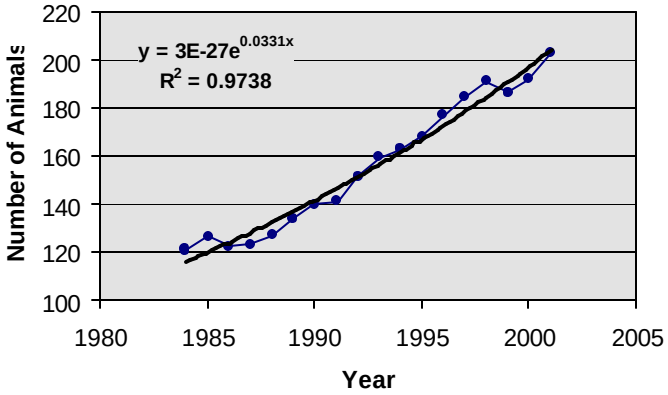
In both the British Columbia northern resident (Olesiuk et al 1990) and Southern Alaskan resident populations the proportion of females that were producing calves declined as a function of age (Figure 11). However in the Alaskan population females produced a larger number of calves later in their lives which was one factor that contributed to the slightly faster growth rate of the Alaskan population.

Figure 11. Proportion of females reproductive as a function of age comparing Prince William Sound/Kenai Fjords with B.C. Northern Residents.



The population trend for Alaskan pods (excluding AB-pod) shows an exponential increase over the study period at a finite rate of 3.3%, (Figure 12) which is similar but slightly greater than the the 2.6% in B.C. northern residents in the 1970's and 1980's (Olesiuk et al 1990). In recent years the northern resident population is static or declining (J. Ford, pers. comm.) while the rate of increase for the Alaskan pods has continued through 2002.

Figure 12. Intrinsic rate of increase for the Prince William Sound/Kenai Fjords resident killer whale population 1984-2001.



The similarity of the age of first viable calf produced and the calving intervals for northern B.C. residents and the resident killer whales in our study indicates that calves are being produced at similar rates in both populations. Since these parameters as well as juvenile survival were quite similar between the populations, we confirmed that is was reasonable to use the same techniques and parameters to estimate ages of Alaskan whales (as described in the Methods). However, the continued calf production later in life of Alaskan females has contributed to a faster rate of increase in the Alaskan population.

Our population model (AB pod excluded) also diverged from the B.C. northern resident model in the lower survival rates in Alaskan female killer whales. This may partly be due to underestimation of the ages of some females because aging was based on physically mature male offspring, however, this bias would be likely in the B.C. data as well (see Discussion). Also, there were a large number of mortalities of apparently older females shortly after the study began in 1986 that may have also contributed to a higher overall mortality rate. However, in Alaska, more adult females than adult males died during this study period (16 versus 11) which is the opposite of what occured in the northern residents. Even so, the sex ratio of adult males to females in Alaskan pods is skewed toward females in any given year (range 1.8 to 1.2; average 1.36), but less so than predicted by the B.C. model (1.63)

The following table compares some of the population parameters we developed for the southern Alaska residents with the same parameters for B.C. northern residents. The longer reproductive lifespan and slightly increased calf output per lifetime for Alaskan residents are factors in the the higher rate of observed increase in the population.

Table 10. Selected population parameters for resident killer whales in Alaska and British Columbia.

REPRODUCTION	***Alaska***	***British Columbia*** (Olesiuk 1990)
First Viable Calf	~15 years	14.9 years
Senescence	45 years	39 years
Reproductive Lifespan	30 years	25 years
Calf Output	5.8 calves	5.3 calves
LIFE EXPECTANCY		
Females	39.4 years	50.1. years
Males	31.4 years	28.7 years
RATE OF INCREASE	3.3%	2.6%
MAXIMUM LONGEVITY		
Females	60-70 years	70-80 years
Males	-	50-60 years

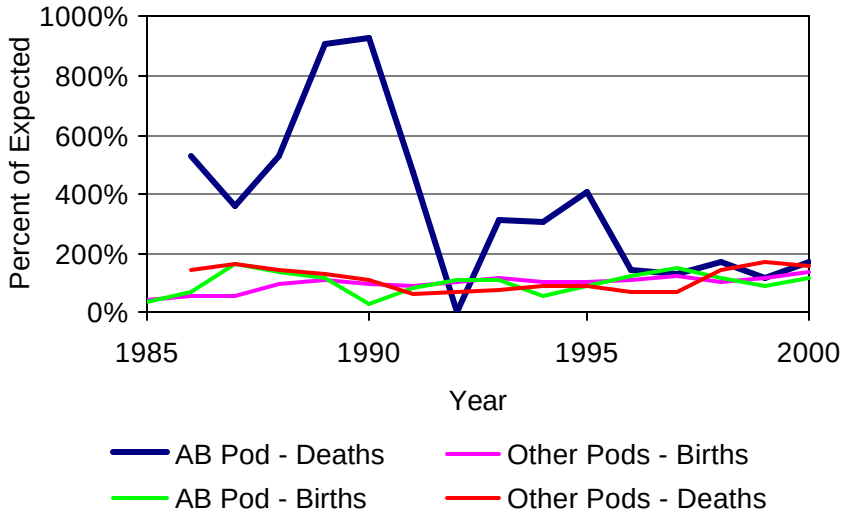
Our population model indicates all pods except AB-pod essentially conform with the B.C. northern resident model (through the mid 1990s), although the intrinsic rate of increase is slightly higher

for the Alaska population and also adult female mortality appears to be higher in Alaska. Since this increase occurs toward the end of the reproductive lifespan, it doesn't have much impact on the productivity of the population and in fact, Alaskan animals have a longer reproductive lifespan and produce more calves resulting in the slightly higher intrinsic rate of increase.

The Alaskan population model predicted that the population should increase at 2.7% per annum and be comprised of 51% juveniles, 23% mature males, 22% reproductive females and 5% post-reproductive females. The population actually grew at 3.3% per annum, and was comprised of 51% juveniles, 19% mature males, 24% reproductive females, and 7% post-reproductive females. The population biology of Alaskan killer whales was remarkably similar to that observed in B.C. and Washington State during the 1970s and 80s, which increased at 2.9% and was comprised of 50% juveniles, 19% mature males, 21% reproductive females, and 10% post-reproductive females. One notable difference was that females in Alaska appeared to experience a more abrupt increase in mortality as they approached reproductive senescence, resulting in reduced longevity and shorter post reproductive lifespan. During the Alaskan study, however, the proportion of post-reproductive females declined from 11% to 5%, suggesting it represented a period of atypically high morality for older females, and as a result we may have underestimated average female life expectancy and longevity.

A comparison of the actual and expected number of deaths in AB-pod indicated mortality for all sex and age classes was much higher than expected based on the model for all other pods over the course of the study (1984-2001). During this study there were 31 deaths in AB pod when only 8.1 were expected. The discrepancy between actual and expected was most pronounced for females and most of those deaths occurred following the *Exxon Valdez* oil spill. During 1989-90, there were 14 deaths in this pod, when only 1.14 would have been expected. The 14 deaths included two juvenile females, six juveniles of unknown sex, four reproductive-age females, three of which had recently matured.

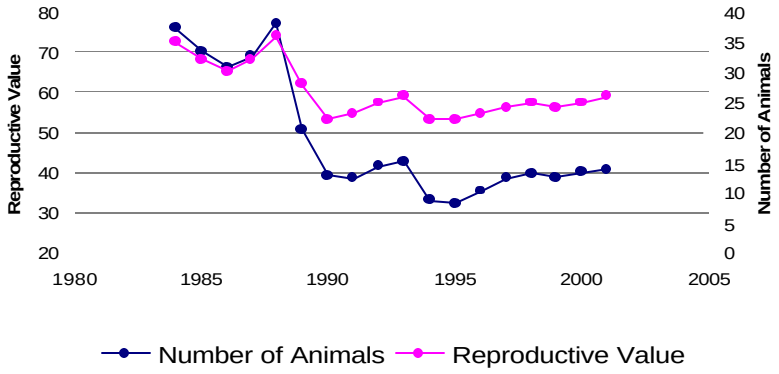
Figure 13. The mortality and reproductive rates as percent of expected for AB pod and all other pods 1984-2000.



The population model indicated that the number of births in AB-pod since 1990 has been close to the number expected based on its sex- and age-structure (14 observed births versus 13 predicted),

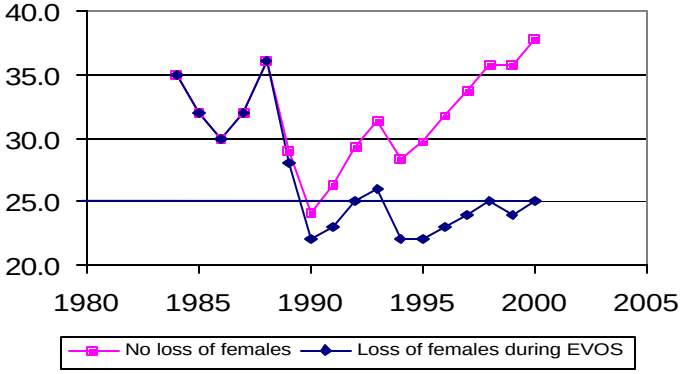
although the number of deaths has been somewhat greater than expected (10 observed versus 6 expected). Thus, a reduced birth rate or greatly elevated death rate does not explain the lack of recovery of AB-pod. However, the reproductive value of the pod declined disproportionately to its reduction in size (49% versus 38% respectively), implying that the animals lost following the spill tended to be those with higher than average reproductive values, i.e. young females. The reproductive potential for AB pod dropped much more dramatically than the number of whales in the pod following the EVOS due to the disproportionate loss of reproductive females (Figure 14). The loss of juvenile females has resulted in the maintenance of this reduced reproductive value.

Figure 14. The number of animals in AB pod compared to the reproductive value of the pod 1984-2001.



Finally, we projected the number of whales that would have been found in AB pod in the years following the spill if there had been the same number of mortalities but no loss of females (using parameters developed in this model). The projection indicated that the pod would have fully recovered and surpassed prespill numbers by 2001.

Figure 15. The number of whales in AB pod compared to the number of whales projected in AB pod had there been no loss of females at the time of the *Exxon Valdez* oil spill.



Discussion

The population biology of Alaskan killer whales was remarkably similar to that observed in British Columbia for the northern resident population during the 1970s and 80s. (Olesiuk et al 1990). Data from the late 1990s suggests the period of growth for the British Columbia northern resident population may have ended. Northern residents peaked in 1997 at 219, then have slightly declined since, to 202 in 2001, then back up to 205 or so in 2002 (J. Ford, pers comm.) while the southern Alaskan resident population continues to grow at a steady rate as of 2002. Another notable difference between populations was that females in Alaska appeared to experience a more abrupt increase in mortality as they approached reproductive senescence, resulting in reduced longevity. During the Alaskan study, however, the proportion of post-reproductive females declined from 11% to 5%, suggesting it represented a period of atypically high morality for older females, and as a result we may have underestimated average female life expectancy and longevity. It is also possible that ages for adult females who were mature at the beginning of the study were underestimated in some cases, which would have created a similar effect. This underestimate of age could occur if males mature at the beginning of the study were judged to be the oldest offspring of reproductive females, when they were actually brothers of these females. Both sons and and brothers may high a high degree of association with a mother or sister (Matkin et al 1999a). However, the genealogies constructed in British Columbia would also be susceptible to this same bias.

Our population analysis suggests that in resident killer whales, there is a relatively steady rate of calf production over time and that mortality rates determine the net gain or loss within the population or pod. Although calf production may vary from year to year due to environmental factors, over an extended period the calf recruitment rate in our region as well as in British Columbia has been quite consistent. Even in the oil spill affected AB pod, recruitment rates considered over the course of the study remained consistent. The pregnancy rate may be substantially higher than the recruitment rate (Olesiuk et al 1990) with calves not surviving in years where the mother is not in good enough condition to support the newborn nutritionally. Pregnancy has a relatively small energetic cost compared to the energetic cost of rearing a calf that may nurse for several years. The long intervals (up to 10 years) between successful calf recruitments for some females may reflect the inability of females to support new calves energetically in many years. In this case, females may have become pregnant, but the calves died at birth or prior to our initiation of fieldwork. Another factor in the increased calving intervals is the the decreased fecundity observed with age.

Populations in both British Columbia (northern resident population) and our area have shown net increases over the past 15 years and may still be recovering from some past perturbation that reduced the population size. Alternately, there may have been an increase in carrying capacity for killer whales in recent decades. In the past, shooting of killer whales may have been a regular occurrence as evidenced in British Columbia by the numerous bullet wounds observed in whales taken into captivity in the 1970s. Also, there was bullet wounding and unexpected mortalities in AB pod during interactions with commercial longline fisheries in the mid 1980s, which may have affected our population model. Although we do not suspect that this was the cause of the unexpected mortalities in AB pod at the time of the spill, this may be an historic factor that reduced the now increasing resident killer whale populations. Additionally, salmon populations were very much reduced from historic numbers prior to the 1970s and salmon are primary prey for these whales at least seasonally (particularly coho and Chinook, see Feeding Habits). It is not unlikely that carrying capacity for resident killer whale has increased in the past 25 years due to recovering salmon populations in British Columbia (until recent years) and particularly in Alaska.

Unexpected mortalities cannot be easily offset by increased calf recruitment, particularly if mortalities include females. From observations in Alaska and British Columbia, we suspect that recruitment rates of around five percent are near the maximum rate for resident killer whales. Despite this potential for increase, AB pod has not recovered from losses at the time of the spill, essentially due to disproportionate loss of females. It is unlikely that recovery will occur for at least another decade. Population characteristics of killer whales (relatively low recruitment rates, lengthy juvenile stage, etc) make rapid recovery from natural or human induced mortality problematic.

GENETICS

Introduction

Genetic subdivision in animal populations is nearly always attributable to physical or behavioural barriers to migration. In only two mammal species that we are aware of, humans and killer whales, is there evidence of fully sympatric populations that are morphologically similar but socially distinct. In humans, ethnic groups may coexist for many generations without fusing. Gypsies, for example, have persisted in the midst of other European groups for centuries. Killer whales in the North Pacific, the focus the study reported on here, 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 this discovery (e.g. Ford 1989, Bigg et al. 1990, Ford 1991, Baird and Dill 1996, Barrett-Lennard et al. 1996a, Matkin et al. 1997). These studies identified individuals based on natural markings, and focused on association patterns and behaviour. It is now evident that the sympatric populations, referred to as *residents* and *transients*, are distinct ecotypes that differ in feeding ecology, behaviour and social organisation (Ford et al. 1998, 2000; Ford and Ellis 1999). Following the Exxon Valdez oil spill is was important to demonstrate the genetic uniquness of resident and transient populations and look for further subdivisions within those populations in order to interpret the effects of whales lost following the spill on their respective populations

Residents prey on fish, principally salmonids. They occasionally harass marine mammals but have not been seen to eat them (Ford et al. 1998). Individuals live in *matrilines*, comprising a matriarch and her complete lineage, both male and female. Matrilines usually contain 4-12 individuals from 2-4 generations, and often travel in association with other matrilines with which they are believed to 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. Each resident pod uses a distinct set of stereotyped calls, or *dialect*; pods with related dialects make up an *acoustic clan* (Ford 1991). Pods associate freely both within and between acoustic clans within their subpopulation but do not associate with pods from adjacent subpopulations (Bigg et al. 1990). Three subpopulations referred to as the *northern*, *southern*, and *southern Alaskan* residents have been studied for many years (numbers and ranges in Table 11).

Transient killer whales live in pods of 1-6 individuals and prey on marine mammals, principally seals, porpoises, and sea lions; they occasionally kill seabirds but have not been seen preying on fish (Ford et al. 1998, Saulitis et al 2000). The membership of a transient pod is often stable for long periods but individuals occasionally disperse between them (Ford and Ellis 1999). As with residents, the transient population is divided into subpopulations with discrete ranges. Studies of transient dialects

are at an early stage, and no equivalent of the acoustic clans seen in residents has been identified. It appears that a similar set of calls is used by all or most members of a subpopulation and that some calls may be shared between subpopulations (Ford 1984, Saulitis 1993). Three subpopulations have been described and are referred to as the *west coast, AT1 and Gulf of Alaska transients* (ranges and sizes in Table 11). The first two of these are sighted frequently; relatively little is known about the third. No association has been seen between transients from adjacent subpopulations.

Recently, a third putative population of *offshore* killer whales has been identified (Table 11). Little is known about this assemblage, other than that it is usually sighted 20 km or more off the coast, ranges between California and the northern Gulf of Alaska, and typically travels in groups of 20 or more individuals (Ford et al. 1994).

Table 11. Estimated size, acoustic clan structure and range of killer whale populations and subpopulations in the northeastern Pacific, and the number of DNA samples from each.

Population	Subpopulation	Abbrev.	Size	Acoustic Clans	Approximate Range	DNA Samples
Resident	Southern	SR	82[1]	J	northern Washington state to southern British Columbia	8
	Northern	NR	214[1]	A, G, R	southern British Columbia to Alaska border	126
	Southern Alaskan	SAR	360[2]+	AB, AD	Alaska border to Cook Inlet	82
Transient	West Coast	WCT	219[3]		central California coast to Icy Bay, Alaska	30
	Gulf of Alaska	GAT	60[3]+		Icy Bay, Alaska, extending west beyond Cook Inlet	8
	AT1	AT1	11[4]		Prince William Sound and Kenai Fjords region	8
Offshore		OFF	200[2]+		offshore waters from central Calfornia to southern Alaska	7

(Acoustic clans of northern and southern residents described by Ford (1991), and of southern Alaskan residents by Jurk et al. (1998). A "+" indicates that not all animals in the subpopulation have been catalogued. [1]Ford et al. 2000, [2]Matkin et al. 1999a, [3]Ford &Ellis 1999, [4]Matkin et al. 1999b.)

Methods

We concentrated our biopsy sampling effort in two areas: in and around Prince William Sound/ Kenai Fjords Alaska (59°30'-61°0'N, 146°15'-151°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). The methods are detailed in "Field Methodolgy" in this report.

Molecular analysis

DNA was extracted from the skin portion of the biopsies by proteinase K digestion, phenol and chloroform purification, and ethanol precipitation using standard procedures (Sambrook et al. 1989). Care was taken to prevent cross-contamination by using sterile disposable labware, flame- or acid-

sterilizing non-disposable items, and using aerosol-filtered pipettor tips during all procedures. DNA extraction and PCR preparations were performed in a laboratory off-limits to amplified PCR products.

Mitochondrial DNA

We sequenced at least one killer whale from each known matriline (based on Ford et al. 1994, Ford & Ellis 1999, and Matkin et al. 1999a) using the following procedure: (1) the entire D-loop region was PCR-amplified using custom-designed primers that annealed to the flanking tRNA-Thr and 12s-rRNA regions (2) the PCR product was purified with QIAQuick® spin columns following protocols supplied by Qiagen, Ltd., (3) a sequencing reaction was performed with Fs-Taq® system reagents and protocols supplied by Applied Biosystems, Ltd., and (4) the sequence was resolved on an Applied Biosystems 377 automated DNA sequencer. Because the sequence was too long (950 bases) to be entirely resolved in one direction, sequencing reactions were run from each end of the amplified fragment. We visually checked the output graphs 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 overlaid each output graph with a reference graph on a transparent sheet, and scanned the two graphs for differences. We then aligned unique sequences using the program CLUSTAL-W (Thompson et al. 1994).

Microsatellites

We tested 27 primer sets developed for microsatellite analysis in cetaceans (Amos 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, 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 amplified product that was similar in size to that described in its original study, we experimentally adjusted the PCR reaction conditions to optimize the selectivity and yield of the reaction. We then used polynucleotide kinase to end-label one of the primers with ^{33}P following standard protocols supplied by New England Biosystems Ltd., and performed PCR under the optimized conditions. The PCR products were resolved on a denaturing polyacrylamide gel and exposed to autoradiograph film. Microsatellite DNA was identified on the developed film by the presence of characteristic shadow bands, and allele sizes were determined 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 believed to be distantly related. 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. 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 compared the two sets of scores.

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 were performed using PAUP* version 4.0b2a, (Swofford 1998). The maximum likelihood analysis used

nucleotide frequencies and transition/transversion ratios based on the sequences. We repeated the analysis on 100 bootstrapped versions of the data to determine support for the tree topology.

Microsatellites

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. The offshores were treated as a seventh subpopulation. 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) Markov chain method as implemented in GENEPOP (Raymond and Rousset 1995). An unbiased estimate of gene diversity (H_e) was calculated for each locus in each subpopulation using Nei and Roychoudhury's formula, (in Nei 1987). 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). To determine 95% confidence intervals for the estimates, we performed 1000 bootstraps by resampling among loci.

We calculated Nei's standard genetic distance D_s (Nei 1972) between all putative subpopulations using MICROSAT 1.5 (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. Stepwise mutation-based measures are expected to be linear with respect to time at phylogenetic time scales, whereas D_s is a more appropriate measure when divergences have taken place recently and genetic drift, not mutation, is the main force creating differentiation (Paetkau et al. 1997). The genetic distance matrix was used to construct a neighbour-joining tree, using the NEIGHBOR subroutine in PHYLIP 3.5c (Felsenstein 1993). To determine support for the tree topology we used MICROSAT to bootstrap the allele frequency data 1000 times by resampling among loci and to calculate distance matrices for each bootstrapped data set. The NEIGHBOR and CONSENSE subroutines in PHYLIP were then used to determine the percentage of bootstraps supporting each part of the tree.

Results

Biopsy Samples

We biopsied 261 identified killer whales off British Columbia and southern Alaska, and obtained tissue from the stranded carcasses of eight additional identified individuals. The population, clan and pod membership of the sampled whales, were from 111 known matrilines and included offshores and members of each resident and transient subpopulation. We also obtained tissue samples from four killer whales from the Atlanticocean.

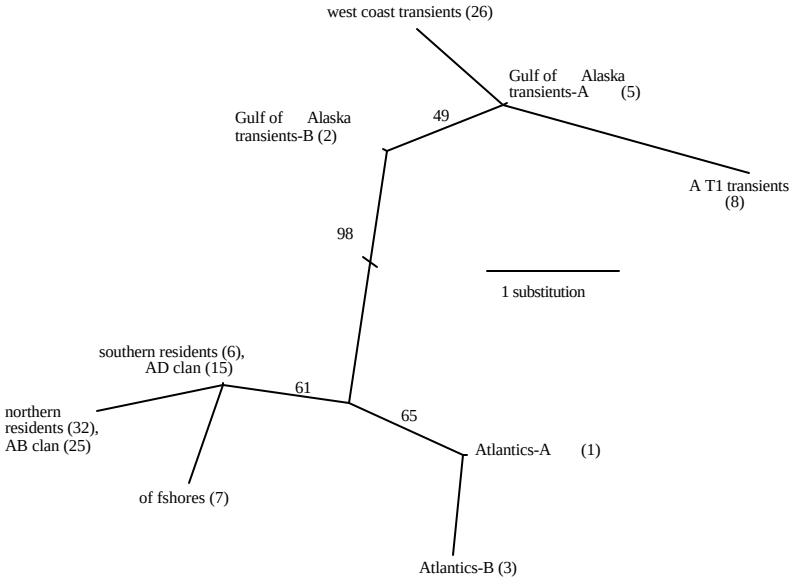
Mitochondrial DNA

A total of 130 killer whales were sequenced. We identified 11 variable sites in the Pacific sequences, comprising one single base-pair insertion/deletion, nine transitions, and one transversion; the Atlantic killer whales added one additional transition. These 12 variable sites defined nine haplotypes.

The northern and southern residents, the AT1 and west coast transients, and the offshores were each monomorphic and had different haplotypes. The southern Alaskan resident subpopulation had two haplotypes, one matching the southern residents, and the other the northern residents; pod members always shared a single haplotype, but pods with different haplotypes were frequently seen in close association. The Gulf of Alaska transients also had two haplotypes, one found in all samples from three pods, the second in both samples from a single pod. Two haplotypes were found in the Atlantic whales, one from a whale that stranded in southern Brazil, the other from two whales captured near Iceland and one that stranded in western France. An unrooted maximum likelihood phylogram based on the D-loop sequence data is presented in Figure 16. The transient subpopulations were an outgroup to all others, including the Atlantic whales. We repeated the mitochondrial analysis with the addition of a Risso's dolphin (*Grampus griseus*) haplotype (Genbank accession number AB018584, contributed by D. Yamagiwa). Separate transient and non-transient monophyletic clades of killer whales rooted by the dolphin sequence were supported by 64 and 56 percent of bootstraps respectively, and 96 percent of the bootstrap trees were monophyletic for at least one of the two killer whale groups.

Figure 16. Maximum likelihood phylogram based on nine killer whale mitochondrial D-loop haplotypes.

The numbers on branches indicate percentage bootstrap support. The number of whales sequenced with each haplotype is shown in brackets. AB and AD refer to two acoustic clans of southern Alaskan residents (Table 11). The suffixes A and B indicate two different haplotypes from the same subpopulation or, in the case of the Atlantics, the same ocean. The length of the longest branch was reduced by half in this drawing (slash mark). In calculating the tree, a single indel in the alignment of the nine groups was accorded the same probability as a T/C transition, however its exclusion from the data did not affect the tree topology. See results for description of rooted tree.



Microsatellite DNA

Five of the 27 primer sets test 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 12). We amplified all 273 DNA samples in the dataset at these 11 loci. Errors were corrected in approximately 1% of the initial scores by re-

scoring. The proportion of missing scores across all loci in the final dataset was 0.004 for biopsied whales and 0.174 for carcasses. 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 (Table 12). Tests for heterozygote deficiency in the northern residents, were negative for all 11 loci, with p values ranging between 0.27 and 0.91. Gene diversity (Table 12) was significantly greater in transients than residents ($F_{1,50} = 12.66$, $p = 0.0008$). Gene diversity in the offshores was similar to the residents, but was not tested statistically.

Table 12. Gene diversities and total number of alleles at 11 microsatellite loci in seven subpopulations of killer whales from Alaska and British Columbia.

Subpop.[2]	FCB4	EV37	FCB12	417	KW2M	FCB17	FCB5	EV1	464	FCB11	415	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[2]	20	9	6	3	8	4	6	7	6	8	9	7.8
Ref.[3]	Buch.	Val.	Buch.	Schl.	Hoel.	Buch.	Buch.	Val.	Schl.	Buch.	Schl.	

([1] Abbreviations as in Table 11. [2] Total number of alleles in all seven subpopulations [3]The original reference describing each locus abbreviated as follows: Buch.: (Buchanan et al. 1996); Val.: (Valsecchi & Amos 1996); Hoel.: (Hoelzel et al. 1998); Schl.: (Schlötterer et al. 1991).)

Estimates of Wright's F-statistics for all seven putative subpopulations, for the three resident subpopulations, and for the three transient subpopulations are presented in Table 13. Population subdivision, or more frequent breeding within subpopulations than expected by chance, is indicated by $F_{st}>0$; $F_{is}<0$ and $F_{is}>0$ indicate that individuals are outbred or inbred with respect to their subpopulations, respectively; and $F_{it}>0$ indicates that individuals are inbred with respect to the total population. Here, the F_{st} estimates reveal strong segregation between offshores, residents, and transients, and weaker subdivision within the resident and transient assemblages. The F_{is} estimates provide no evidence that inbreeding occurs within the subpopulations. Pairwise F_{st} estimates are presented in Table 14. Figure 17 is a neighbour-joining phylogram of the seven subpopulations based on their genetic distances.

Table 13. Weir and Cockerham (1984) estimators of F-statistics combined over 11 microsatellite loci for killer whale subpopulations from Prince William Sound, Alaska and British Columbia[†].

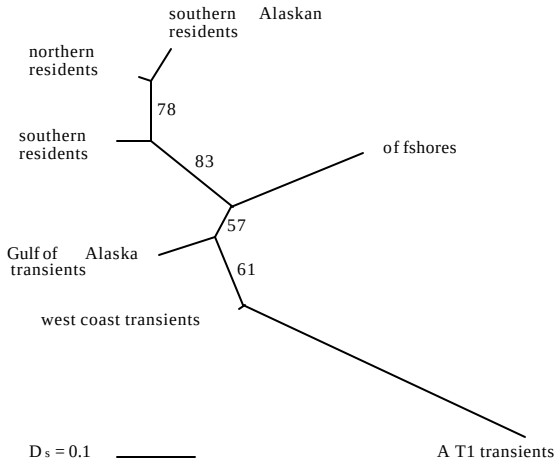
	*F*is	*F*st	*F*it
all subpopulations [7]	-0.014 (-0.049 — 0.022)	0.205 (0.140 — 0.269)	0.194 (0.114 — 0.276)
resident subpopulations [3]	-0.019 (-0.056 — 0.020)	0.088 (0.032 — 0.146)	0.070 (0.003 — 0.127)
transient subpopulations [3]	0.004 (-0.096 — 0.086)	0.167 (0.088 — 0.241)	0.170 (0.073 — 0.236)

([†] Subpopulations as listed in Table 11. Round brackets indicate 99% confidence intervals; square brackets the numbers of subpopulations in each analysis.)

Table 14. Weir and Cockerham (1984) estimators of *F*st combined over 11 microsatellite loci 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. (Abbreviations as in Table 11. For testing for *Fst* differences from zero, multi-locus genotypes were permuted among subpopulations 10,000 times.)

Residents	NR	0.144					
	SAR	0.187	0.076				
Offshores	OFF	0.321	0.278	0.305			
Transients	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
			Residents		Offshores	Transients	

Figure 17. Unrooted neighbour-joining phylogram for Alaskan and British Columbian killer whales based on 11 microsatellite loci, using Nei's standard genetic distances.



The numbers give percentage bootstrap support. When the offshore population was removed, support for the resident/transient separation was 97%. Atlantic killer whales were not included in this analysis because of their small sample size.

Discussion

Our 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: the number of samples that we collected and analysed was several times greater than in any earlier study; all Pacific killer whales included in the study were positively identified; four of the six subpopulations analysed here had not been compared previously; at least one whale was biopsied from as many matrilines as possible (previous studies used multiple samples from a small set of matrilines); and the length of mitochondrial DNA sequenced and the number of microsatellite loci typed were substantially greater than in earlier studies. Our findings have five significant implications (expanded on below) that are either novel or more conclusive than in earlier studies.

1. Resident and transient killer whales are reproductively isolated.

Individuals classified *a priori* as resident and transient had no mitochondrial haplotypes in common, and there were many more fixed mitochondrial differences between the two populations than among their subpopulations (Figure 16, also see Stevens et al. 1989 and Hoelzel et al. 1998). Since the classifications were made independently of any genetic comparisons and our samples were large, we are confident that female migration between the two forms has been extremely rare for many generations. 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 the general patterns are similar: the microsatellite phylogram (Figure 17) preserves the separation of residents and transients, pairwise *F*st values (Table 14) are much higher between resident and transient subpopulations than between subpopulations of a common population, and several loci have population-specific alleles. These results suggest that neither sex disperses at an appreciable rate between populations.

There is no reason to suppose that residents and transients are reproductively incompatible. Both have crossed with Icelandic whales in captivity (mating records from Duffield et al. 1995, whale origins from Hoyt 1984) and produced fertile offspring. Since residents and transients are sympatric, their genetic separation must be maintained by positive assortative mating. Mating preferences could be based on culturally or genetically inherited behaviours that distinguish residents and transients, such as those associated with foraging (e.g. Morton 1990, Barrett-Lennard 1996a, Ford et al. 1998) or communication (Ford 1991). They could also be influenced by subtle differences in phenotype (see Bigg et al. 1987, Baird & Stacey 1988). However, it seems unlikely that mating preferences alone could account for the genetic isolation of the two populations. We argue below that the social cohesion of subpopulations is likely the most important factor in the isolation of residents and transients.

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

Our finding of fixed mitochondrial differences between the northern and southern residents effectively rules out substantial female-mediated gene flow between them, and confirms the pattern reported by Hoelzel (1991) and Hoelzel et al. (1998). The microsatellite analysis showed that they are strongly differentiated at nuclear loci as well, 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 southern resident 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. There have also been sightings of northern residents in areas normally frequented by southern residents. Members of the two populations must come into acoustic and perhaps visual

contact at least occasionally, indicating that their reproductive isolation results from behavioural or social factors rather than physical separation.

The southern Alaska residents have two mitochondrial DNA haplotypes, one in common with the southern and the other with the northern residents, suggesting that they share relatively recent maternal ancestors with both groups. Their microsatellite genotypes indicate relatively weak separation from the northern residents and much stronger separation from the southern residents, as reflected in the *F*st values in Table 14 and the bootstrap values in Figure 17. These patterns may reflect contemporary patterns of gene flow, with occasional matings taking place between the southern Alaskan and northern residents but few matings between either population and the southern residents, or they may reflect historical associations and founding events. The only observation of possible association between resident subpopulations was a sighting of two pods of southern Alaska residents in proximity to two northern resident pods (Dahlheim et al. 1997). In contrast, associations among pods from the same subpopulation are seen very commonly.

The general pattern of genetic differentiation among transient subpopulations is similar to that of residents. The four transient mitochondrial haplotypes cluster more closely with each other than with the haplotypes of any other population with strong bootstrap support (Figure 17). At the same time, the fixed sequence differences between transient subpopulations suggest that female dispersal between them is rare at best. The microsatellite-based pairwise *F*st estimate for the west coast and Gulf of Alaska transient subpopulations is relatively low, evidence that their separation is either incomplete or has occurred recently. The separation of both groups from the small AT1 transient subpopulation appears to be older and/or more complete. The isolation of the AT1's appears likely to result in extinction, as it presently numbers fewer than 15 and has not produced surviving offspring for 16 years (Matkin et al. 1999b).

he subdivision of both residents and transients into genetically differentiated parapatric subpopulations cannot be explained by mating preferences associated with divergent feeding behaviours or phenotypes, and suggests that subpopulations are cohesive social units, not simply collections of individuals sharing a common range. Cohesion requires that individuals reliably distinguish members of their social unit from non-members. In killer whales recognition is likely based both on direct encounters between individuals and on acoustic contact. Killer whales move up to 170 km per day and are capable of communicating acoustically over distances of at least 10 km (unpublished data); it is therefore likely that every member of the subpopulation encounters every other member frequently.

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

The terms resident and transient were first applied to killer whales in British Columbia (Bigg et al. 1982). The same terms were later used to classify killer whales in the Prince William Sound region of Alaska because of obvious behavioural and ecological parallels (Leatherwood et al. 1990). Since neither resident nor transient killer whales have been known to move between the two areas, 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 that each group had a single origin. The initial divergence could have occurred sympatrically or allopatrically. However, the data described here suggest that the divergence is now widening in sympatry because reproductive isolation appears to be complete.

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

Residents and offshores probably share more recent maternal ancestors with each other than either does with transients, based on their similar mitochondrial DNA haplotypes (Figure 15) and on the

rooting of the mitchondrial tree with a Risso's dolphin outgroup. We found the opposite pattern at microsatellite loci, where offshores and transients were most similar (Figure 17). This situation is consistent with three scenarios of historical and contemporary gene flow, as follows: (1) offshores diverged from ancestral residents but have occasionally mated with non-offshore males, usually transients (2) offshores diverged from ancestral transients and experienced mitochondrial DNA introgression after one or more resident females emigrated into the group (3) the offshore divergence preceded that of residents and transients and was followed by occasional hybridisation with both populations. 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 exclusively within their subpopulation (Barrett-Lennard et al. in prep.), we suspect that the first scenario is most likely.

5. Residents remain within their natal pods for life and have lower levels of genetic variation than transients.

One of the most striking findings to emerge from nearly 30 years of field studies of resident killer whales is the absence of dispersal of members of either sex from their natal matrilines (Ford et al. 2000). Here we asked whether the lack of dispersal over this period is typical of the recent history of the population. The southern Alaska resident group consists of pods belonging to two 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, and paternity analysis indicates that inter-clan matings are common (Barrett-Lennard et al. in prep). If females dispersed between pods even rarely, the observed relationship between clan membership and mitochondrial haplotype would break down. We conclude therefore that successful dispersal by female residents has not occurred for many generations. Mitochondrial comparisons cannot detect historical trends in male dispersal, but can identify males that have themselves dispersed between subpopulations. In accordance with field studies, no male dispersers were found genetically.

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 increase the relative fitness of group members. Amos (1999) offered an alternative explanation: 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 mitochondrial DNA variation in transients than in residents (four haplotypes in three subpopulations and two haplotypes in three subpopulations, respectively). This finding is consistent with both hypotheses since both link mtDNA variability to dispersal, and transients, unlike residents, disperse between pods (Ford & Ellis 1999).

Microsatellite DNA diversity was also significantly higher in transients than in residents. This difference may indicate that the mean subpopulation size of transients is larger than that of residents. Although more residents than transients have been photo-identified and catalogued, transients are more difficult to census than residents (Ford & Ellis 1999), and many west coast and Gulf of Alaska transients may remain uncatalogued. Transient subpopulations may also be less closed to gene flow than residents, and their genetic diversity may be augmented by occasional matings with either offshores or unknown subpopulations of killer whales. Finally, the patterns could result from historical contingencies— recent bottlenecks or founder effects—in residents. Barrett-Lennard et al. (in prep.) rule out a fourth explanation, that matings between close kin are frequent in residents since they do not disperse from their natal groups.

<u>Sympatric origin of population subdivision</u>

We have shown that killer whale populations in the northeastern Pacific show a remarkable amount of structure in the absence of physical boundaries–not only are ecotypic populations separated, but each is strongly subdivided. This structure appears to be maintained by a strong behavioural tendency for individuals to avoid associating with members of other subpopulations. Since subpopulations are relatively small (average resident effective subpopulation size is approximately 70, assuming that all females of reproductive age and 1/3 of mature males breed), periodic inter-group mating should help to maintain variation and to restore beneficial alleles lost to mutation and drift. Presumably, associating with non-group members has historically had attendant costs that outweighed these advantages. While the nature and extent of these costs is conjectural, they plausibly include risks of aggressive conflict, resource competition during the period of association, future competition arising from the transfer of local knowledge, and disease transmission. These costs, however, likely apply to many other social species that do not show sharp sympatric and parapatric population subdivision and thus are not wholly satisfying in explaining the patterns seen in killer whales.

We suggest that the ability of killer whales to maintain long-term traditions, particularly vocal traditions (Ford 1991), reduces the disadvantages and increases the advantages to individuals of remaining within their subpopulation. Killer whales advertise their presence through the use of culturally-inherited dialects (Ford 1991). This makes reliable recognition possible, as discussed above, allowing both kin-selected and reciprocally altruistic behaviours to develop (Trivers 1971). These behaviours should reduce the likelihood of interference competition or other conflict between related groups, and could also foster co-operative resource defence. Barrett-Lennard et al. (in prep) showed that dialect similarity and probability of mating are negatively correlated within resident subpopulations, suggesting that vocal traditions allow individuals to avoid inbreeding while remaining in their subpopulations.
We propose that the creation of new killer whale subpopulations results from the fission of large subpopulations in the following manner. (1) When a subpopulation expands its range beyond a critical size, member pods that usually forage at different extremes of the range encounter each other less and less often, and eventually cease to recognize each other as members of the same subpopulation. (2) A range boundary forms between the groups of pods as their social separation becomes complete. (3) Pods that usually forage in the central part of the ancestral range initially associate with both of the groups but are eventually drawn into one of them, completing the isolation of the new subpopulations. The initial divergence of residents and transients could have occurred in a similar manner, prior to the development of feeding specialisations. The formation of new groups in this way is expected to result in greater levels of genetic variance among groups than would be the case if new groups formed from migrants drawn from the population at large (Whitlock & McCauley 1990), which may explain the high *Fst*s in Table 13 relative to those reported in other social mammals (summarized in Storz, 1999).

There has been much interest in the role in speciation of learned dialects in birds (e.g. Baptista & Trail 1992), but little consideration of the possibility of speciation arising from culturally-transmitted traditions in mammals. Killer whales appear to be good candidates for such consideration. Residents and transient killer whales occupy separate ecological niches, and do not interbreed, even in sympatry. They are separate species now by Simpson's (1961) evolutionary definition, and barring demographic or environmental catastrophes, there is no obvious impediment to them becoming biological species sensu Mayr (1942) over time.

ACOUSTICS

Introduction

In recent years we have used acoustic recognition of killer whale populations and resident pods to track numbers and movements of killer whales during winter months using remote hydrophones. In order to accomplish this, first, the dialects of the different populations and groups within these population and their relationships had to be recorded and described and compared with the results of our genetic analysis (see GENETICS). In this section present the background analysis that was necessary to interpret the data collected from remote listening stations Cultural traditions are tools to conserve information for several generations without transcribing the information into the genetic code. The advantage of cultural traditions over genetically transmitted information is that cultural traditions can adapt faster to changes in the environment (Cacalli-Sforza and Feldman 1981; Boyd and Richerson 1985).

The discrete call types of killer whales that we report on here are likely the result of cultural traditions. Cultural traditions have been implicated in a number of observations of recurring behaviours in a few other mammals and in many birds, where they often involve vocalizations (Mundinger 1980). Learned vocal traditions include song types, phrases or notes produced by many songbirds (Marler and Tamura 1962; Slater and Ince 1979; Payne et al. 1985; Trainer 1989) as well as song types and themes of humpback whale songs, *Megaptera novaeangliae* (Payne et al. 1983), discrete calls produced by killer whales (Ford 1991), and discrete temporal patterns in click vocalizations of sperm whales, Physeter macrcephalus (Weilgart and Whitehead 1997). Most of these traditions are commonly called dialects (Connor 1982).

Dialects in killer whales and sperm whales are believed to function as social identification markers of groups that continuously mix (Ford 1989; Weilgart and Whitehead 1997), and are therefore different from dialects that occur in geographically isolated populations. Most dialects that fall into the second category are epiphenomena that result from cultural mutation and drift, and are therefore selectively neutral with regard to biological evolution (Williams and Slater 1992; Lynch 1996, p.181: Payne 1996, p.198). However, social dialects appear to be culturally selected and could therefore play a role in the biological evolution of densely packed animal societies (Baptista 1975; Conner 1982; Ford 1991; Weilgart and Whitehead 1997), e.g. dialects could reinforce assortative mating in order to avoid inbreeding or outbreeding depression (Treisman 1978).

The social organization, behaviour, and vocalizations of resident killer whales off the coast of British Columbia have been studied for the last 27 years (e.g. Ford et al. 2000). This resident killer whale population consists of groups of closely related animals (matrilines). Neither male nor female killer whales appear to disperse from their matriline, and all matrilines use specific dialects as vocal signatures (Bigg et al. 1990; Ford 1991). Matrilines that associate often are considered to be closely related and are called pods (Bigg et al. 1990). Pods share a repertoire of 7-17 discrete calls, which appears not to change considerably over several generations (Ford 1991).

Bigg et al. (1990) suggested that pod fission occurs gradually and over the course of several generations. According to this hypothesis, 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 change progressively and repertoires diverge. This implies that pods with very similar repertoires have

split more recently and are more closely related than pods that have fewer calls in common. Ford (1991) termed pods that share parts of their repertoires vocal *clans*.

Different clans have entirely distinct call-type repertoires. A resident killer whale community consists of pods, often from different clans, but pods of the same clan always belong to the same community. For example, the 'Northern Resident' community, which ranges from central British Columbia to Southeast Alaska consists of three clans, called A-, G-, and R-clan, while only one clan, J-clan, forms the 'Southern Resident' community that occurs in the waters of Washington State and Southern British Columbia. Our study examined the discrete call in relation to population in the southern Alaska populations of killer whales.

Vocalizations of resident killer whales

Vocalizations of killer whales fall into three categories, *clicks*, *whistles* and *calls*. *Clicks* are heard in 95% of all encounters with residents, and 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 (Ford 1989; Thomsen 1998). After echolocation clicks, discrete *calls* are the most common type of vocalization, which is also the type that forms dialects.

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. 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) . Deecke (1998) investigated the evolution of call variants and found a relationship between acoustic change and the degree of association between different matrilines.

Calls are highly repetitive and stereotyped pulsed vocalizations. The repetition rates of pulses, which are reflected in the distance between harmonic contours seen in the spectrogram (see Watkins 1966), are usually modulated over the call's duration. Calls have distinct tonal properties because of high pulse repetition rates. Many calls contain silent intervals as well as abrupt shifts in pulse repetition rate often accompanied by changes in sound pressure. These intervals and shifts allow the call to be divided into different parts and elements. In accordance with Miller and Bain (in press), components that were produced by higher sound and pulse-repetition rates (first band starts above 2 khz and harmonics are widely spaced) were called upper frequency components (uf-component), and the ones with the lower sound and repetition frequencies (first band starts usually below 2 kHz and harmonics are spaced closer together than in uf-component) were called lower frequency components (lf-component).

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

Calls were collected, identification photos taken and skin biopsies obtained as described in "Field Methods" of this report. We only analyzed recordings of each pod when it was encountered alone or at

such a distance from other pods 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). All recordings meeting the above criteria were used to describe the call repertoire of a pod.

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. Samples of recognized calls (minimum of 15 per pod) were digitized and later analyzed using Canary, Version 1.2.4 (Cornell Laboratory of Ornithology 1998).

The calls that we used for spectrographic analysis were digitized at a 44.1 kHz sampling rate with a 16 bit sample size. The spectrographic analysis was done using frame lengths of 1024 points for each analyzed time series, which resulted in a frequency filter bandwidth of 174 Hz. A 1024 point Fast-Fourier-Transformation (FFT) of time series with an overlap of 87.5% for consecutive series resulted in spectrograms with 2.9 ms time resolution and 43 Hz frequency resolution.

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). 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), independently, obtained similar call categories from two captive killer whales of the same population that Ford (1984) analyzed using an by-ear and visual spectrogram inspection for call classification. Furthermore, Deecke (1999) used neural networks to discriminate between calls and came to similar distinctions as any of the above by-ear and visual classifications. Call-types therefore are based on their *gestalt* appearance and can be adequately described through means of human perception. We also gave call samples to two other researchers familiar with killer whale vocalizations for re-classification.

Discrete 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. The appendices i, ii, iii etc. that were used in combination with some call types indicate the existence of sub-types.

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), we only assigned sub-types when calls were consistently recorded in several different contexts.

We obtained a quantitative measure of the similarity of call repertoires for each pair of pods 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.

Results

Different observers made 848 recordings parallel to photo-identification of the whales between 1984 and 1999. (Table 15) We analyzed 112 single pod recordings that were distributed over the whole recording period. The recordings for each of the seven pods ranged from 16 to 22, while the biopsy samples ranged from 2 to 8 reflecting the number of matrilines in each pod. In total, 9000 calls were classified by ear and spectrographically inspected.

Table 15. Pod encounters with analyzed recordings of six pods and number of biopsy samples collected from these pods in each year. Actual recording duration differed among encounters, so did vocal activity.

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	

The energy distribution within the call spectrum usually allowed good spectrographic representation of frequencies from 0.5 kHz to 12-14 kHz. A number of calls had two simultaneously appearing contours, the so-called upper (uf-component) and lower frequency components (lf-component). The lf-components ranged in frequency from 0.5 to 4.5 kHz, while the upper fundamental frequencies ranged from 2 to 11 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 higher frequencies. Uf-components were more likely to be seen in the spectrogram when vocalizing whales were moving towards the hydrophone. 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.

Call description and classification

Most call-types could be easily distinguished by ear from one another. They usually differed in most of the acoustic parameters chosen for this description: number and duration of parts and elements, as well as peak frequency and repetition rate (contours) changes between elements. Call types by pod are listed in Table 16.

More than one part was found in 8 call-types: AKS- 01,03,08,11,13.14,17,22. The initial part was in all cases characterized by low repetition rates that sometimes could have been confused with a string of echolocation clicks. However, these low repetition pulses always preceded another pulse

sound of higher repetition frequency by equal or less than 0.1 seconds. In five of these eight calls, 01, 03, 11, 17, and 22, the following part differed in number of elements and/or contour modulations between pods. Call types 13 and 14 did not show great variations in either element structure or contour modulation between pods, AB, AI, and AN.

Two call types, AKS 08 and ASK 10 were characterized by their high number of parts or elements. AKS 08, was characterized by 2 to 6 parts that had identical contours. The contours only differed in duration between the initial and the following parts. However, the repetition rates of all parts differed between pods. While AKS 08s produced by AB and AI pod had repetition rates that clustered around 2000 cycles per second, produced AN pod the same call with a repetition rate of 4000 cycles per second. AKS 10 was produced by AB, AI, AJ and AN pod, and was characterized by the longest duration (> 2.5 sec.) and highest number of elements (up to 6) of all call-types.

Four call-types, AKS 03, 09, 11, and 22 had prominent pairs of Lf- and Uf-components, which allowed an easy classification of the similarity of calls produced by different pods. Call-type AKS 05 produced by AD, AE, and AK pod and AKS 07 produced by AB, AI, AJ, and AN pod consisted of an Uf-component alone, and therefore appeared acoustically more similar to a whistle than to any other pulsed call. Variation between pods was minimal.

The call-types AKS 04 produced by AD, AE, and AK pod, and AKS 15 produced by AB, AI, AJ, and AN pod were characterized by their low repetition rates and low peak frequencies, as well as their small degrees of contour variation. These two call-types were predominantly recorded in situations when the majority of the whales in a group were *resting*.

AKS 02 is one of the 9 call types that were not shared by whales from more than one pod. The other 8 call-types were 06, 18, 20, 21 23, 24, 27, and 28. All of these calls were distinct with regard to the number of elements and contour variation.

Table 16. 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.

Pods/Types	AB	AI	AJ	AN	AD	AE	AK
AKS 01 i					X		X
ii					X		X
iii					X		
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		
AKS 20					X			
AKS 21						X		
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

The seven pods - AB, AD, AE, AI, AJ AK, and AN pod produced a total number of 39 calls. These calls could be placed into 26 categories of distinct types according their number of parts, number of elements, and contour modulations. Ten of these 26 distinct types appeared as more than one stable variant or sub-type. Overall, one of the ten types appeared as 4 sub-types, one as 3 sub-types, and eight as 2 sub-types. AN pod and AD pod have recently undergone fission into four pods called AN10, AN20, AD5 and AD16 (Matkin et. al. 1999). However, because a great number of recordings that we analyzed were form times when these pods were still considered associating closely. Therefore, we did not make a distinction into four pods in our analysis.

Subtypes

Sub-types predominantly varied in the amount of elements and/or showed differences in the contour variation of elements of calls produced by different pods. Calls, such as AKS 17 and AKS 01 that were characterized by simple contour modulations, usually down-sweeping contours, produced more sub-types than calls that were structurally more complex, such as AKS22 or AKS03 that had prominet shifts in their contours. Generally, a pod only used one sub-type of a call. Therefore, if sub-types existed that could often be used to distinguish pods. However, there were three cases in which sub-types existed in the repertoire of only one pod, AKS01 in AD pod, AKS02 in AE pod and

AKS24 in AJ pod. These pods were characterized by matrilines that swim often alone (AD5 and AD 16) or by pods that shared the least amount of calls with other pods (AE and AJ).

Call demographics

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. 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 traveling, 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.).

Call sharing

Approximately 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. 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*.

No calls or sub-types were shared between clans. Overall, more sub-types were shared than calls without variants, and the maximum number of sub-types of a particular call was often equal to the number of pods that shared that call, e.g. four sub-types of AKS 17 used by AB, AI, AJ, and AN pod. 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 acoustic similarity index (Table 17). Because pods from different *clans* did not share any calls the acoustic similarity between them was 0. 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 are displayed in the form of a dendrogram by means of single-link cluster analysis (Morgan et al. 1976).

Table 17. 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.

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

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 (Table 11). 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 (Table 11). Killer whales of this community can usually be found in Southern British Columbia, Washington State and occasionally further south.

Discussion

Alaskan residents appear to use their dialects to reduce chances of mating between relatives. 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 . Barrett-Lennard et al. (in prep.) compared micro-satellite DNA within and between clans of the Northern Residents in British Columbia and found that mating occurs mainly between whales that are acoustically dissimilar as revealed by Ford (1991).

Alaskan resident pods like their counterparts further south have been observed to mix often with other pods during the summer months. Although sexual behaviour during these gatherings has only been observed between members of the same sex (Rose 1992) mating is commonly thought to take place (Bigg et al. 1990). Matings most likely occur underwater. Male/female pairs from different pods have been observed spending periods of time in close association with each other, and longer dives are common during these pair associations (Bigg et al. 1990). 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.

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 1989). Ford (1991) also

noted that call repertoires of some pods had remained relatively constant for more than 25 years. The ability to learn to reproduce only the repertoire of its own pod makes it very likely that an individual whale knows all of the existing discrete calls of associating matrilines. Furthermore, because of the non-dispersal of either sex from the matrilines the call repertoire of each matriline is very resilient against changes. Therefore, even after matrilines split the vocal repertoire stays similar for several generations. Repertoire sharing produces vocal clans, which then help residents to identify whales that they are related to but not associate very often. Furthermore, because of the existence of clans and the knowledge of the shared repertoires residents are culturally different from the transient and offshore whale population. This cultural difference appears to function as a breeding barrier between these populations.

In comparison with other dolphin populations, residents belong to very small breeding populations. The population estimates for Alaskan residents consistently stayed below 400 whales (Matkin et al. 1999b), and the effective population size is even smaller than expected, because only a certain number of adult whales reproduce (L.B.-L., pers.comm.). This increases the probability for inbreeding considerably. In addition, all resident killer whales specialize on fish as their diet, and on salmonids as their main target (Ford et al. 1998; Saulitis et al. 2000). Therefore, residents are behaviourally distinct from other killer whale populations. This specialization has likely been developed by a small group of killer whales within a fish-eating population, and might have been the cause for segregation from that population. Foraging on salmonids requires knowledge of where, when and how to find prey and an ability to quickly adapt to fluctuations in salmon migrations over time. Therefore, one would expect residents A) to be able to learn and memorize information well, and B) having developed mechanisms that reduce chances of inter-mating between closely related individuals.

That vocally learned dialects are advantageous adaptations and play a role in the gene flow within and between populations is still controversially discussed (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). Some of this lack of consensus might may arise from the fact that repertoire differences between geographically separated populations cannot have a social function. In contrast, social dialects that occur within breeding populations should be considered in tests of adaptive functions. Many 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). However, this does not explain the prevalence and long stability of dialects among groups in mixing populations (e.g. Ford 1991; Weilgart and Whitehead 1997). In these situations, social dialects are most likely culturally selected and may function as a possible reinforcement in assortative mating to avoid inbreeding depression.

Although experimental evidence that dialects in killer whales are vocally learned does not exist (Janik and Slater 1997), 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.

Therefore, residents appear to use the culturally selected trait, a dialect selectively learned within the matriline, to determine the degree of relatedness of a possible mate. Therefore, the group specific

dialect of residents functions in similar ways than incest taboos function in human communities (Durham 1991).

A similar model for cultural selection of dialects and how they could have influenced the distribution of genes among human populations was suggested by Hill (1979). Hill proposed 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). If this model were true than the similarity in dialect evolution between the resident killer whales and humans has to be based on similar ecological needs. Within early human societies the evolution of languages appears to be correlated with the development of different farming techniques (Renfrew 1989).

We suggest that the dialects of resident killer whales are culturally selected traditions to reduce the negative effects of inbreeding and that the ability to make use of dialects in this way has arisen from the need to make effective use of a spatially and temporally fluctuating abundance of an energy rich food source, the salmon.

REMOTE HYDROPHONE

Introduction

In order to determine identities of groups from remote recordings of killer whales calls, it is necessary to develop an all inclusive call catalogue and attribute those calls to specific groups. In previous reports (Matkin et al. 1998; Matkin, et al. 1999; Matkin, et al. 2000; Matkin, et al. 2001; Matkin et al. 2002) we examined the pod specific call repertoires of nine pods, AB, AD16, AE, AF, AG, AI, AJ, AK, and AN10. These pods[1] form two distinct vocal clans in the Southern Alaska resident community (SAR), the AB-clan (AB, AF, AG, AI, AJ and AN pod) and the AD-clan (AD16, AE, and AK pod). Calls are not shared between clans (Yurk et al. 2002) and repertoire exclusiveness of vocal clans is matched by genetic differences of the mitochondrial and nuclear DNA (Barrett-Lennard 2000). In our last report (Matkin, Ellis et al. 2002), we presented the results of a test of human inter-observer reliability in recognizing killer whale call-types. The results of this test showed that qualitative structural analysis using both the sound and the spectrogram (an optical representation of the sound) is a valid method to classify killer whale call types and repertoire similarities (Yurk, Barrett-Lennard et al. 2002).

In this report we present the extent of all known repertoires of 11 well known southern Alaskan pods, which include those mentioned above and the repertoires of AD5 and AN20 pod. We also present some results of preliminary analyses of the repertoire(s) of another 'group' of matrilines, the AX 'group'[2]. This call catalogue was then used to analyze recordings from a remote listening station in

[1] The term pod has recently been challenged as an accurate description of a distinct social unit, and should therefore be replaced by the term matriline (J. Ford pers. comm). As a result the term pod is used here as a substitute for matrilines that share a particular call repertoire. Call repertoires of pods are not completely distinct because call types are shared among them. Call type structure, however, appears to be distinct on the pod level.

[2] The AX group might not be one pod with a homogenous social structure, but might consist of several different social units that are not necessarily related (G.Ellis, pers. comm.).

Resurrection Bay, where a majority of the pods that were recorded between January to March 2001 and October to December 2001 were identified.

Methods

Since 1996 we analyzed the call repertoires of the following 9 pods. Table 18 provides an overview of the number of recordings entered in this long-term study since 1984. The number of identified call types is greater than 10,000. Analytical techniques are based on those used in Yurk et al. (2002) and Matkin et al. (2002) and are also summarized in the Acoustics section of this paper.

To describe vocal similarity between groups, we obtained a measure of the similarity of call-type repertoires or dialects for each pair of pods from an index based on the degree of call-type sharing. This index was derived from Dice's coefficient of association (Ford 1991), which normalizes the data to account for differences in repertoire size:

$$\textit{Index of Similarity} = \frac{2N_C}{R_1 + R_2}$$

where N_C is the total number of call-types and sub-types shared, and R_1 and R_2 are the repertoire sizes (call-types plus subtypes) of the two pods. We used the index values, which ranged between 0 – 1, to calculate a hierarchical structure of acoustic similarity, which we displayed in the form of a dendrogram by means of average-link cluster analysis.

Table 18. Number of pod encounters with recordings analysed for 11 pods (AB through AK) for each year. Actual recording duration differed among encounters, as did vocal activity.

year/pod	AB	AF	AG	AI	AJ	AN*	AD**	AE	AK	# of recs./ year
1984	9	4	0	3	1	4	4	3	2	30
1985	4	1	0	0	0	2	2	4	1	14
1986	0	0	0	0	0	1	0	0	0	1
1988	0	0	0	0	0	0	0	0	1	1
1989	2	0	0	0	0	1	0	0	3	6

1990	1	0	0	3	4	2	1	2	2	15
1991	0	0	0	3	1	2	1	3	2	12
1992	3	0	0	2	0	1	0	0	1	7
1993	0	0	0	0	0	0	1	0	0	1
1994	0	0	0	1	1	0	0	0	0	2
1995	5	2	0	1	1	1	0	3	1	14
1996	1	0	2	0	1	0	0	1	0	5
1997	0	0	4	1	2	4	4	3	2	20
1998	1	0	1	0	0	0	1	0	1	4
1999	0	0	1	0	0	0	1	0	0	2
2000	3	2	1	1	4	1	5	2	3	22
2001	2	4	3	0	2	1	4	0	4	20
total # of recordings	31	13	12	15	17	20	24	21	23	176

* Both AN10 and AN20

** Both AD5 and AD16

Results and Discussion

Call type classification of Southern Alaskan Resident (SAR) pods

We analysed 176 of 329 pod recordings that were distributed over the whole recording period (Table 18). The number of recording sessions per pod ranged from 12 to 31 with durations of sessions ranging from 5 to 135 minutes. A minimum of 120 minutes of recording was inspected for each pod. In total, 10,000 calls were categorized by listening to the calls and visually inspecting the corresponding spectrograms.

Table 19. List of all identified call types of 11 resident killer whale pods and their variants in alphanumerical order.

An X in the appropriate column indicates call types produced by an individual pod. Pods that share call types are grouped together. The R indicates a call type that is mainly produced during times when the groups are resting.

call#/pod	AB	AI	AJ	AN10	AN20	AF	AG	AX	AK	AD16	AD5	AE
AKS 01 i									X	X		
ii									X	X		
iii											X	
AKS 02 i												X
ii												X
AKS 03									X	X	X	X
AKS 04 (R)									X	X	X	X
AKS 05									X	X	X	X
AKS 06												X
AKS 07											X	
AKS 08											X	
AKS 09 i									X	X		
ii									X	X	X	
iii											X	
AKS 10 i	X	X	X	X	X			X				
ii	X	X		X	X	X		X				
AKS 11 i	X	X	X									
ii	X	X	X	X	X	X	X					
AKS 12	X											
AKS 13 i	X	X		X	X	X						
ii	X			X								
AKS 14	X	X										
AKS 15 i (R)	X	X		X	X	X	X					
ii (R)	X	X		X	X							
iii (R)			X									
AKS 16 i						X	X					
ii						X	X					
AKS 17 i	X	X				X						
ii	X	X										
iii	X			X				X				
iv		X		X								
AKS 18	X	X	X	X	X							
AKS 19 i						X	X					
ii						X	X					
AKS 20					X							
AKS 21 i	X	X		X	X							
extinct ii	X											
AKS 22	X	X	X	X	X							
AKS 23			X									
AKS 24 i	X		X									
ii		X	X									
iii			X									
AKS 25			X									
AKS 26			X									
AKS 27							X					
AKS 28						X						
AKS 29						X						
AKS 30								X				
AKS 31 i								X				
ii								X				

AKS 32								X				
AKS 33								X				
AKS 34								X				
AKS 35										X		
AKS 36												X
TOTAL(54)	18	15	12	12	10	11	7	9	7	7	8	6

The 11 pods AB, AD5, AD 16, AE, AF, AG, AI, AJ AK, AN10, AN20 and the AX group produced 36 distinct call-types. However, call repertoires of AF, AG and AX might be larger because considerably fewer recordings of those groups had been analyzed than of the other 9 pods. Twelve of the 36 distinct types exhibited more than one stable variant. One type had four stable variants, four had three variants, and seven had two variants making a total of 54 discrete calls. Of these 54 discrete calls one (AKS 21ii) has to be considered extinct because it did not appear in any recording after 1990. Table 19 lists all discrete calls and the pods that produced them.

The mean number of call types produced by each pod was 10 (sd =3.96), while the median was 9 call types. AB pod had the most call types, 18, and AE the least call types, 6. Both the AG pod and the AX group repertoire might be underestimated due to low number of recordings.

The vocal relationships among the 12 groups, which is given by repertoire similarity index (Table 20) is depicted in Figure 18 as a dendrogram reflecting the vocal relatedness. The vocal relatedness has been shown to demonstrate an accurate measure of the maternal relatedness in Alaskan resident killer whales.(Yurk, Barrett-Lennard et al. 2002).

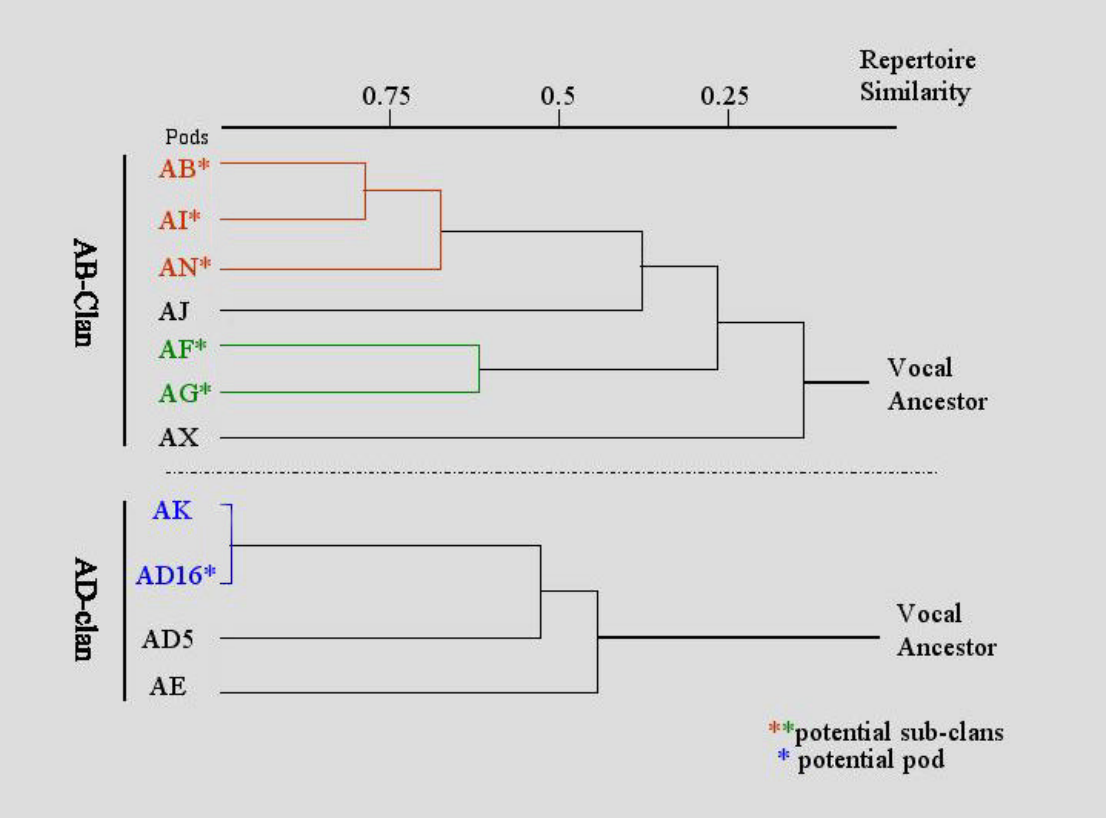
Table 20. Acoustic similarity between pod repertoires based on the index of similarity (Ford 31991), where 1 means the repertoire of two pods are identical and 0 means the two pods do not share any call.

	AB	AI	AN	AJ	AF	AX	AG	AK	AD16	AD5	AE
AB	1										
AI	0.788	1									
AN	0.645	0.714	1								
AJ	0.467	0.444	0.32	1							
AF	0.357	0.4	0.348	0.091	1						
AX	0.222	0.167	0.273	0.095	0.105	1					
AG	0.167	0.19	0.211	0.111	0.625	0	1				
AK	0	0	0	0	0	0	0	1			
AD16	0	0	0	0	0	0	0	1	1		
AD5	0	0	0	0	0	0	0	0.533	0.533	1	
AE	0	0	0	0	0	0	0	0.462	0.462	0.429	1

AN pod has recently started travelling as two pods called AN10 and AN20 (Matkin, Ellis et al. 1999) who were encountered more often alone or in company of other pods than with each other. The call repertoires of the two sister-pods, however can still be considered identical. Thus, we did not distinguish between the pods in our repertoire similarity analysis. To assess call type usage differences between AN10 or AN 20 accurately and thus determine repertoire divergence between the sister pods

we need to collect more single recordings of both sister-pods in the future . AD pod appeared to have also split into 2 sub-pods, AD16 and AD5. The call repertoire relatedness between the 'sister'-pods however, is lower than between the AD16 and AK pod. We, therefore assume that the original assignment of the two pods as AD pod might have reflected only temporal travelling relationships between the two pods, AD16 and AD5 during early years of the study. Based on repertoire sharing the dialects of AK and AD16 pod are identical. However, there exist structural differences between identical call types produced by these two pods. Furthermore, the frequency with which certain calls are produced is quite different. While AK uses mainly AKS01i and AKS09i, AD16 uses predominantly AKS01ii and AKS09ii. The two sister pods also spent considerable time together.

Figure 18. Degree of repertoire similarity between pods based on an average-cluster dendrogram of acoustic similarity.



Analysis of remote hydrophone recordings

The remote hydrophone at Thumb Bay in Resurrection Bay was monitored on 127 days between February 23, 2001 and May 25, 2001 (break during summer boating season) and October 3 and December 31, 2001 for a total of 972 hours and 25 minutes. Of that time, killer whales were heard

on 40 days for a total of 147 hours and 55 minutes. On 38 days and in 43 separate sessions 29 hours and 15 minutes of recordings were made from killer whales. The days and time of recordings and the recorded call-types with acoustically identified pods are presented in Table 21.

Table 21. Call types and groups identified from remote hydrophone recordings 2001.

Date	Whales present/ recorded (min)	Recognized Call Types (AKS)	Pods (Groups) present
March 02,2001	355/90	11i,11ii,14,18,22, 23, 24i	AB, AJ, AN
March 05, 2001	160/90	11i,12,17i,17iii, 17iv, 21i, 22	AB, AN, (AI)
March 06, 2001	500/135	11i,11ii,12,14,17i, 17iii,17iv,2122, 24i	AB, AN
April 09, 2001	110/45	01iii,03,05,09iii, 2 new calls	AD5 + unkown.group
April 10, 2001	830/10	01iii,05,09iiii	AD5
April 24, 2001	35/35	01iii,09iii,2new calls	AD5 + unkown group
April 27, 2001	30/30	01iii,09iii	AD5
April 28, 2001	250/50	01iii,[01iv-new variant],03,09iii, [09iv-new variant], 2 new calls	AD5, unknown group
May 02, 2001	150/90	01iii,03,05,09iii	AD5
May 04, 2001	95/30	01iii,03,05,09iii	AD5
May 07, 2001	85/15	01i,05,09i,09ii	AK, [AD16]
May 08, 2001	325/45	01iii,[01iv-new variant],03,09iii, [09iv-new variant], 2 new calls	AD5, unknown group
May 09, 2001	175/20	01iii,[01iv-new variant],03[new variant],09iii, [09iv-new variant],	AD5, unknown group
May 10, 2001	35/10	01iii,[01iv-new variant],03,09iii,	AD5, unknown group
May 11, 2001	25/15	01iii,09iii	AD5
May 14, 2001	165/35	01iii,[01iv-new variant],03,09iii, 2 new calls	AD5, unknown group
May 22, 2001	50/10	01iii, 01i,	AD5, AK
May 23, 2001	100/90	01iii, 09ii, 09iii	AD5, AK or AD16
Field Season			
Date	Whales present/ recorded (min)	Recognized Call Types (AKS)	Pods (Groups) present

Oct 14, 2001	45/45	very noisy	not determined
Oct 15, 2001	45/45	01i, 09i	AK
Dec 05, 2001	205/45	11i,11ii,12,13i,14,17ii,17iv,23	AB, AJ, AN
Dec 06, 2001	285/30	11ii,13,14,18,23, 24i	AB, AJ, [AN]
Dec 07, 2001	155/30	11i,11ii,13,14,17i,17ii,18,23	AB, AJ, [AN]
Dec 08, 2001	190/15	10ii,14,17i,18,23, 24i	AB, AJ, [AN]
Dec 09, 2001	575/15	11ii,13,14,18,21i 23	AB, AJ, [ANand/orAI]
Dec 14, 2001	245/45	11ii,13,14,18,23	AB, AJ
Dec 15, 2001	140/90	11i,11ii,12,13,14, 18,23	AB, AJ
Dec 16, 2001	340/65	11i,11ii,12,13,14, 17i,18,21i,22,23	AB, AJ, AN
Dec 17, 2001	135/25	11i,11ii,12,13,14, 17i,18,19i, 21i,22,23, 28	AB, AF, AJ, AN
Dec 18, 2001	100/45	11i,11ii,12,13,14, 17i,18,19i, 21i,22,23, 28	AB, AF, AJ, AN
Dec 19, 2001	165/45	11i,11ii,12,13,14, 17i,17iv,21i,22,23	AB, AJ, AN
Dec 21, 2001	200/45	11i, 11ii, 17i	AB or AI
Dec 22, 2001	250/45	11i, 11ii,14,17i, 17ii	AB
Dec 23, 2001	590/90	12,13,14,17i, 18, 21,28	AB, AF,[AN]
Dec 24, 2001	460/45	11i,11ii,13,14,17i, 18,19ii,21i,22,28	AB, AF, AN
Dec 25, 2001	235/35	11i,11ii,14	AB
Dec 26-27, 2001	45/10	11i,11ii,12,13,14, 17i,17ii,17iv,19ii, 21i,22,23	AB,AJ,AN

* Because of a low signal-to-noise ratio we could not determine which pods were present on Oct 14 . The pods in parentheses could also have been present given the call types recognized. However, the call structure allowed the conclusion that the pods outside of parentheses were present.

Conclusions

The vocal similarity among AB clan members allows a further division of the pods into four partially distinct vocal groups (sub-clans) with less than 50% repertoire similarity among them. According to the results AB, AI, and AN form a sub-clan (called AB sub-clan after the largest repertoire group) and AF and AG form the AF sub-clan. The AJ pod is a distinct pod that might have close relatives, which have not been identified yet. The AX group appears not to be a single pod but could be comprised of several more closely related sub groups that sometimes travel together (Ellis,

pers. comm.) Using the same criteria AD clan is comprised of one sub-clan, the AD sub-clan consisting of AD5, AD16, and AK pods, from which the AE pod is acoustically distinct. Within the AD sub-clan AD5 is vocally only distantly related to AK and AD16.

Barrett-Lennard (2000) showed that in contrast to the Northern Residents in British Columbia and Southeast Alaska where matings occur exclusively between members of different clans, some matings among Southern Alaskan Residents occur between members of the same clan. However, those matings are all between members of acoustically only distantly related pods, such as between members of the AB sub-clan (AB, AI, and AN pods) and members of AJ pod, and possibly AF sub-clan (AF and AG pods) or the AX group.

Considering the results presented in this report with the results of analyses reported earlier of recordings from remote hydrophones in Prince William Sound and in Resurrection Bay (Matkin, Ellis et al. 1999; Matkin, Ellis et al. 2000; Matkin, Ellis et al. 2001; Matkin, Ellis et al. 2002) it appears that during winter months only pods of the same clan associate. Prey availability might be considerably reduced during the winter months, which could result in a segregation of un-related pods to increase inclusive fitness. Mating appears to take place mainly during the late summer and predominantly between members of different clans (Barrett-Lennard 2000).

Remote acoustic monitoring or sensing appears to be a highly effective method to trace movements of killer whales, particularly resident killer whales, during winter months. On the 59 days that killer whales were recorded the group identities were determined 58 times. On only 8 days were whales present that could not be identified acoustically. This was probably due to a lack of field recordings of certain member groups of the Southern Alaskan Residents. We would like to increase the number of remote monitoring stations at so-called killer whale abundance 'hot-spots'. However, installation, maintenance, and monitoring carry a high cost and are very time consuming. Nevertheless, we feel that the monitoring program is a very important research tool because it allows us to determine habitat use of killer whales during times when fieldwork is impossible. Better knowledge of the habitat use of an apex predator will help us better understand trophic relationships within an ecosystem.

FEEDING HABITS

Introduction

Killer whales have been reported to feed on nearly every marine mammal species available to them throughout their cosmopolitan range (Hoyt 1984; Jefferson *et al.* 1991; Matkin and Saulitis 1994). Off the coast of British Columbia and Washington State, they have been observed to feed on seventeen species of fishes and squid (Ford *et al.* 1998). Once thought to be opportunistic predators (e.g., Rice 1968), recent findings suggest that some populations of killer whales exhibit dietary specializations. Two sympatric, non-associating populations of killer whale, known as *resident* and *transient*, have been identified in our study area as well as in other regions of the North Pacific and have been separated by their unique feeding habits; resident killer whales feed exclusively on fishes and squids; transients feed exclusively on mammals (Ford et al 1998) Two sympatric, non-associating forms of killer whale have also been identified in Prince William Sound, Alaska (Ellis 1987; Heise *et al.* 1992) and our genetically unique (see GENETICS, this report). These forms conform closely in behavioral characteristics to those identified off the coasts of British Columbia (Bigg *et al.* 1987; Morton 1990;

Ford *et al.* 1994). At least three populations of killer whale, two of the transient type and one of the resident type, have been proposed for Prince William Sound based on DNA analysis (Matkin *et al.* 1998), social characteristics (Saulitis 1993; Matkin and Saulitis 1994) and acoustics (Saulitis 1993; Barrett-Lennard *et al.* 1996; E.S., unpubl. data). Prince William Sound resident killer whales travel in social groups called pods, containing seven to 36 related individuals (Matkin *et al.* 1994; in press.). As is the case for British Columbia resident pods (Bigg *et al.* 1990), Prince William Sound resident pods exhibit long-term stability, with no immigration or emigration of members (Matkin *et al.* in press). Fourteen resident pods totaling 461 individuals in 1997 have been identified in Prince William Sound (Matkin *et al.* in press). Of these, 202 individuals in nine pods are considered regular visitors (Matkin *et al.* in press).

Residents use Prince William Sound waters most frequently during July, August, and September (Matkin *et al.* 1997), though they appear to make occasional visits to the area year-round (Matkin *et al.* 1998). Prince William Sound residents have been sighted as far west as Kodiak Island (Matkin *et al.* 1997). While residents from southeastern Alaska have been seen in the Sound, Prince William Sound residents have not been documented east of the Sound (Matkin *et al.* 1997).

At least two separate populations of transient killer whales use Prince William Sound: the AT1 and the Gulf of Alaska transient populations. Both populations travel in small groups that are more fluid in size and individual membership than are resident pods (Matkin *et al.* 1994). AT1 transients typically travel in groups of two to four individuals but occasionally travel singly or in groups of ten or more individuals (Saulitis 1993). In 1997, the AT1 transient population contained 11 individuals (Matkin *et al.* 1998) but currently numbers only 9 individuals.

The Gulf of Alaska transients travel in groups of two to eight individuals and have not been seen together as a single assemblage or intermingling with AT1 transients (Matkin and Saulitis 1994). The Gulf of Alaska and AT1 transients are distinguishable by differences in mitochondrial DNA and nuclear DNA (Matkin *et al.* 1998) and acoustic characteristics (E.S., unpubl. data).

Members of the AT1 transient population have been sighted year-round in Prince William Sound (Matkin and Saulitis 1994) and in Resurrection and Aialik Bays, west of Prince William Sound (Matkin *et al.* 1998). The Gulf of Alaska transients are seen infrequently in Prince William Sound; their range is unknown, though they have been seen as far west as the Kodiak Island waters (unpubl. data).

Potential marine mammal prey in Prince William Sound are Dall's porpoises, harbor porpoises, humpback (*Megaptera novaeangliae*), minke (*Balaenoptera acutorostrata*) and gray (*Eschrichtius robustus*) whales, harbor seals, Steller sea lions and river (*Lutra canadensis*) and sea otters (*Enhydra lutris*). Pacific herring and five species of Pacific salmon are found in Prince William Sound. Various species of bottom fish, including Pacific halibut and sablefish (*Anoplopoma fimbria*), are common.

Materials and Methods

Dietary and behavioral data were gathered concurrently with census data collected during this study. Although months spent in the field varied among years, data collection occurred during July and August in all years of the study.

Harassment was considered to have occurred when potential prey animals exhibited an avoidance or alarm response in the presence of nearby killer whales or when killer whales chased, followed or lunged at potential prey without making a kill, or when, following an attack, a kill was suspected but could not be confirmed.

Marine mammal kills were confirmed by the observation of marine mammal parts in the mouths of the whales, bits of blubber, skin, viscera, hair, and/or blood in the water and/or oil on the surface in the vicinity of the whales. The species identity of marine mammal prey was usually determined during observations of attacks and chases. Fish predation was confirmed by observations of fish in the mouths of whales or by fish scales in the water at the kill sight.

When successful predation was suspected, the kill site was approached slowly. An observer on the bow of the research vessel scanned the area and retrieved fish scales or other prey fragments using a long handled dip-net. Samples were placed in envelopes labeled with the date, time, location of the kill site, and the identity and/or pod designation of the animal making the kill. Scale samples were identified to species at the Fish Aging Laboratory, Pacific Biological Station, Nanaimo, B.C.

Results

The data presented here represent 662 encounters with killer whales from 1984-1996, 196 of which were with transients and 466 of which were with residents. Residents and transients were never seen together in the same encounter.

The AT1 population was the most commonly seen transient population (n= 174 encounters). Gulf of Alaska transients and unclassified transients were seen rarely in Prince William Sound during the study (n = 22 encounters).

Residents spent significantly more time resting than did transients (Table 22). Residents spent more time socializing than transients, and transients spent more time foraging than residents, though these differences were not significant. Residents and transients spent nearly equal amounts of time traveling. Both residents and transients spent a large proportion of their time traveling and foraging (70% and 89%, respectively).

Table 22. Percentage of time spent in each activity state and p-values for ANOVA's run on each activity state for resident and transient killer whales in Prince William Sound, Alaska, 1988-1996.

	Rest	Travel	Forage	Socialize
Transient	4.1	38.5	50.0	7.4
Resident	17.6	35.2	35.5	11.7
p-value	0.0019	0.0645	0.078	0.0823

Killer whales used three distinct foraging strategies during this study: open water foraging for mammals, nearshore foraging for mammals, and foraging for fishes. Only transient killer whales were seen open water and nearshore foraging for marine mammals.

During open water foraging for mammals, whales were generally farther than one km offshore. When hunting at the surface, the whales milled or traveled slowly, and movements of individual whales were not synchronized. The whales traveled for a km or more beneath the surface at times, often during dives of ten-minute or longer duration. When prey was detected, a coordinated chase involving all whales in the group ensued, and prey was shared among group members.

During nearshore foraging for mammals, whales generally remained within 20 m of shore. Individuals typically separated from one another, exploring different parts of the shoreline.

Only residents were observed foraging for salmon. During this type of foraging, echolocation clicks were heard and the whales were often dispersed widely over several square kilometers.

Thirty-one kills of marine mammals by transient killer whales were documented. Transients preyed almost exclusively upon Dall's porpoises and harbor seals (Table 23). Only one other species, the harbor porpoise, was documented as prey. Most of the unidentified marine mammals preyed upon by killer whales (n = 7) were described as unidentified porpoises (n = 4); the remaining prey items were unidentified marine mammals (n = 2) or unidentified pinnipeds (n = 1).

Table 23. Diet of transient killer whales in Prince William Sound, Alaska based on thirty-one documented kills, April-October, 1984-1996.

Prey Species	#Killed
Phocoenoides dalli	12
Phoca vitulina	10
Phocoena phocoena	2
Unidentified mammal	7

Most harbor seal kills (n = 11) occurred beneath the water's surface and were detected by the appearance of blubber fragments and oil on the surface. Seabirds often investigated these sites and somtimes alerted us to their presence. In contrast, Dall's porpoises kills involved highly visible surface chases. All but three harbor seal kills occurred during nearshore foraging, and all Dall's porpoise kills occurred during offshore foraging. Transients spent 21.5% of their time nearshore foraging and 23.8% of their time offshore foraging, indicating that they spent nearly an equal amount of time hunting for seals as for porpoises.

Forty-three harassments of marine mammals by transient killer whales were documented (Table 24). Most harassments were of Steller sea lions (n = 14) and harbor seals (n = 22). Of the fourteen Steller sea lion harassments, four were by AT1 transients and ten were by Gulf of Alaska transients. All harbor seal kills and harassments documented in this study were made by AT1 transients.

Table 24. Harassments of potential prey by transient killer whales.

Species	Total	AT1	GOA
Harbor seal	22(28.2)	22(36.1)	0
Dall's porpoise	6(13.9)	4(13.3)	2(15.4)
Stellers sea lion	14(32.6)	4(13.3)	10(76.9)
Humpback whale	6(13.9)	6(20)	0
Sea otter	3(7.0)	2(6.7)	1(7.7)
River otter	1(2.3)	1(3.3)	0
Salmon	1(2.3)	1(3.3)	0

Total	43	30	13

On 11 occasions, two individuals of the Gulf of Alaska transient population were observed in the vicinity of the Steller sea lion haul-out at the Needle, in Montague Strait, in southwestern Prince William Sound. Although kills were not observed, during all of these observations, Steller sea lions appeared agitated and were harassed by the whales. Transient killer whales were never observed preying on fish; however, in one instance, an AT1 individual chased a salmon beneath the research vessel.

Sixty-three scale samples were collected from fish kills made by resident killer whales in five years of the study (1991-2; 1994-6). Ninety-five percent of the samples were from coho salmon (Table 25). The rest of the samples were from chinook (*O. tshawytscha*) and chum (*O. keta*) salmon. Twelve samples were collected from unidentified resident whales. About half of the scale samples (n = 29) were collected in August. On 38 occasions, predation on fish by resident killer whales was observed but scale samples were not collected. Thirty-six of these kills were of salmon, one was of herring and one was of halibut. In recent years predation on Chinook salmon has been observed regularly in May and June in Kenai Fjords (C Matkin pers.obs.)

Table 25. Salmon species preyed on by resident killer whales in Prince William Sound, July-September, 1991-1996 based on analysis of sixty-three scale samples collected from individual killer whales (41 photo- identified; 10 identified to pod; 12 unknown) representing seven pods.

Pod	*#O. kisutch*	*#O. tshawytscha*	*#O. keta*
AB	14	0	0
AN	2	1	0
AI	3	0	0
AE	20	0	0
AJ	4	0	0
AK	3	0	2
AD	1	1	0
unknown	12	0	0

Resident killer whales interacted non-aggressively with marine mammals on 66 occasions, 47 of which involved Dall's porpoises and 16 of which involved Steller sea lions. Interactions with a humpback whale, a minke whale and a sea otter were documented on single occasions. The baleen whales were observed feeding among resident killer whales for extended periods of time. Dall's porpoises were observed swimming with resident killer whales, engaging in play behaviors with killer whale calves, and surfacing rapidly just in front of killer whales, sometimes making physical contact. One individually recognizable Dall's porpoise remained with the AB resident pod from May through September in 1984. Steller sea lions interacted with residents on 13 occasions by surfacing among them, porpoising towards them or by nipping at them. Interactions occurred during all four general killer whale activity states, occurred from April through September, and involved all resident pods.

Discussion

This study confirmed that resident and transient killer whales in Prince William Sound exhibited distinct dietary preferences, as Ford *et al.* (1999) found in residents and transients off British Columbia and Washington State. Transients in Prince William Sound were observed feeding exclusively on

marine mammals, while residents were observed feeding exclusively on fish. The fish-eating and mammal-eating forms occurred sympatrically, but did not associate.

Stomach content analyses from a variety of regions suggest that killer whales consume either fish or mammals and not both (Nishiwaki and Handa 1958; Betesheva 1961; Berzin and Vladimirov 1983; Bigg *et al.* 1990; Ford *et al.* 1999). The stomach contents of five killer whale carcasses recovered in or near Prince William Sound reflect the same pattern of feeding segregation (Barrett-Lennard *et al.* 1995; Heise *et al.* in prep.). Stomach content data reported for British Columbia reflect the same pattern: no stomachs that contained mammal remains also contained fish remains (Ford *et al.* in press).

Ford *et al.* (in press) summarizes observations of predations and stomach contents of stranded killer whales from 1975-1995 from the coastal waters of British Columbia, Washington State and southeastern Alaska. Transient killer whales preyed upon seven species of marine mammal. Fifty-five percent of the 130 predations were of harbor seals and only 5% were of Dall's porpoises (Ford *et al.* in press). Ford *et al.* (in press) reported a 90% success rate for transients attacking harbor seals, while Baird (1994) reported a 100% success rate for harbor seal attacks off southern Vancouver Island. Over 95% of 136 kills observed by Baird (1994) were of harbor seals. Harbor seal numbers in British Columbia, southeastern Alaska and Washington State have been increasing exponentially since 1970 (Olesiuk *et al.* 1990). In areas of high harbor seal population numbers, they appear to be the preferred prey for transients. The comparatively low and declining number of harbor seals in Prince William Sound and the Gulf of Alaska may have caused transients to shift to Dall's porpoises as their preferred prey. Dall's porpoise attacks are much more vigorous than those of harbor seals, lasting up to 43 minutes (this study) and involving high speed chases with aerial leaps. Dall's porpoise attacks have lower success rates (39%: Ford *et al.* in press) than harbor seal attacks.

In the Gulf of Alaska and Aleutian Islands, harbor seal counts during the molting season declined by 19% from 1989-1995 (Hill *et al.* 1996). The most recent population estimate of harbor seals in Prince William Sound is 5,300 (Frost *et al.* 1996). Harbor seals in Prince William Sound are continuing to decline at an estimated rate of 5% per year (K. Frost, pers. comm.). Native hunters from Chenega Village, in southwestern Prince William Sound, report a drastic decline in harbor seal numbers in the study area (M. Eleshansky, pers. comm.).

The observations of feeding behavior in this study were strongly biased by season. Most observations were made during the summer months. It is probable that prey distributions and transient feeding behavior change seasonally as well as geographically. Observations by reliable observers indicate that juvenile Steller sea lions become more abundant in Prince William Sound with the arrival of herring in early spring (R. Corcoran; D. Rand, pers. comm.). These observers have documented transient killer whales from the Gulf of Alaska population preying upon Steller sea lions during early spring months (Barrett-Lennard *et al.* 1995; Heise *et al.* in prep.). Barrett-Lennard *et al.* (1995) estimated that Steller sea lions make up 15% of the diet of transient killer whales in Alaska. Steller sea lions make up 6% of the diet of transient killer whales off British Columbia, Washington State and southeastern Alaska (Ford *et al.* in press).

In our study, some members of the Gulf of Alaska transient population were consistently observed harassing Steller sea lions around the Needle, a haul-out in southwestern Prince William Sound. A Gulf of Alaska transient carcass contained 14 Steller sea lion tags. Although members of the AT1 population were seen to harrass Steller sea lions on occasion, they were never seen foraging around Steller sea lion haul-outs or attacking or preying upon Steller sea lions. None of carcasses identified as belonging to the AT1 population contained sea lion remains.

Steller sea lion predation may involve considerable risks to killer whales due to the large size and aggressive nature of adult sea lions. Off British Columbia, Steller sea lion attacks often lasted for 1-2.5 hours before the prey was killed (Ford *et al.* in press). Steller sea lions were observed charging toward both resident and transient killer whales in this study.

Some killer whale populations may specialize on particular prey species, especially when successful capture requires highly developed hunting skills and substantial risk to the whales. Killer whale calves off the Crozet Archipelago learn from their mothers the technique of intentional stranding, a highly risky behavior that sometimes results in killer whale mortality (Guinet and Bouvier 1995). Harbor seal predation in Prince William Sound may likewise require intricate local knowledge of the coastline and location of harbor seal concentrations to efficiently locate prey.

Nonetheless, data on harassments of marine mammals by killer whales suggest that the diet of transient killer whales in Prince William Sound is more diverse than what is reflected in the observations of kills. There have been reliable reports of killer whales attacking humpback whales in Prince William Sound (N. Naslund, P. Kompkoff, pers. comm.). Our observations of harassments of humpback whales by transient killer whales indicate that this species may be a component of the transient killer whale diet.

It is probable that harbor porpoises make up a larger percentage of the diet of transient killer whales than is reflected in our data, since very little of our field effort occurred during times of harbor porpoise abundance. The abundance of harbor porpoises in Prince William Sound appears to fluctuate seasonally, with numbers decreasing during the summer months (pers. obs.). Harbor porpoises make up 12% of the diet of transient killer whales off British Columbia, Washington State and southeastern Alaska (Ford *et al.* in press). Ford *et al.* (in press) reported a 100% success rate for harbor porpoise attacks.

While direct comparisons among activity budgets of killer whales from different areas are not feasible due to observer bias and variations in definitions of behavioral categories, the overall trends in differences between residents and transients in Prince William Sound are similar to those observed off British Columbia and Washington State (Ford 1989; Morton 1990; Baird 1994; Felleman *et al.* 1991). For example, Morton (1990) compared the behavioral budgets of resident and transient killer whales off the central British Columbia coast. Transients foraged and traveled more than residents, and residents socialized and rested more than transients. Results of the present study exhibited a similar, though not significantly significant, trend.

In the case of both residents and transients, traveling and foraging behavior are difficult to distinguish, and may, in fact, overlap in function. Most killer whale activity during foraging and traveling occurs beneath the water's surface. In all areas where they have been studied, residents spend 58-72% of their time traveling and foraging, while transients spend between 88.5-94.5% of their time traveling and foraging (Ford 1989; Morton 1990; Baird 1994; Felleman *et al.* 1991; this study).

Transients spend less time resting than residents in all areas where they have been studied (Ford 1989; Morton 1990; Baird 1994). Group resting behavior in resident killer whales is a highly coordinated activity that may help to reinforce the strong social bonds within resident pods (Jacobsen 1986, 1990; Osborne 1986). Transient killer whales have a more fluid group membership, and for them, group resting may not have the same social significance. Resting behavior in transients may also occur after dark, when prey are more difficult to locate visually.

Though resident killer whales off the coasts of British Columbia and Washington State prey upon all six species of Pacific salmon, they appear to prey preferentially on chinook salmon (Ford *et al.* in press). Chinook salmon are rare in southwestern Prince William Sound during July and August (S.

Morestad, pers. comm.). The presence of chinook salmon scales in our sample is therefore significant. Chinook salmon are by far the largest and most energetically rich of the five Pacific salmon species found in the Gulf of Alaska. Large runs of chinook salmon enter the Copper River Delta adjacent to Prince William Sound in May and June. Commercial fisherman report large groups of killer whales off the Copper River during that time (D. Bilderback, pers. comm.), while few resident pods are encountered in Prince William Sound during the same months. In addition, chinook salmon are present in Prince William Sound year-round (S. Morestad, pers. comm.).

Scale sample collection and observations of predation in southwestern Prince William Sound suggest a strong seasonal selectivity by resident killer whales for coho salmon in July and August. Selectivity for coho salmon by resident killer whales during the summer months in Prince William Sound is not surprising. Coho salmon are the second largest of the five salmon species found in Prince William Sound, and contain the third highest amounts of protein, fat, and calories (Sidwell 1981; Exler 1987; Groot and Margolis 1991). In addition, coho salmon are present in nearshore waters from May through December (S. Morestad, pers. comm.), and thus provide a consistent food source for most of the year.

In British Columbia waters, however, similar methods yielded few samples of coho scales, even though coho are more abundant there than in Prince William Sound (Ford *et al.* in press), suggesting that, where chinook salmon are abundant, they may be the preferred prey species of resident killer whales. In addition, in British Columbia chinook salmon are a year-round prey source for resident killer whales (Ford *et al.* in press).

Sockeye salmon contain the second highest amount of fat of the five Pacific salmon species (Sidwell 1981; Exler 1987). While they contain a comparable amount of fat per fish and are available from May through July in Prince William Sound, no sockeye predation was documented in this study. Off British Columbia and Washington State, sockeye salmon make up only 4% of documented predations (Ford *et al.* in press). Recently we have observed extensive feeding on Chinook salmon in spring in Kenai Fjords (C. Matkin, pers.obs.)

Pink salmon are the smallest and lowest in fat content of the five Pacific salmon species (Exler 1987; Sidwell 1981). While they comprised 17% of scale samples collected at sites of killer whale predation off British Columbia and Washington State (Ford *et al.* in press), there was no pink salmon predation documented in Prince William Sound, despite extremely large returns of pink salmon. A bias against the collection of pink salmon scales may exist since the scales are much smaller than those of other species and may be more difficult to observe in the water. It seems more likely, however, that since coho salmon are present in the Sound at the same time as pink salmon, because of their higher energy content and larger size, they are the preferred prey species.

While five species of Pacific salmon are found in Prince William Sound, 95% of scale samples were of coho salmon. The high caloric content of coho salmon and their availability for many months of the year may have resulted in Prince William Sound resident killer whales adapting their foraging strategy for coho salmon and not for other salmon species, while killer whales in British Columbia may have adapted their foraging strategies for chinook salmon for the same reasons.

Our data, however, reflect only those fish kills that were made at the surface. While there is little information on the vertical distribution of salmon at sea, coho prefer the highest minimum ocean temperatures, between 5-5.9 C, which typically occur at the surface (Groot and Margolis 1991).

Different prey choices among populations of killer whales are accompanied by different foraging strategies and social structure. For example, killer whales off both Argentina and the Crozet Archipelago, in the southern Indian Ocean, have adopted the technique of intentional stranding in order

to capture pinnipeds at haul-out sites (Lopez and Lopez 1985; Hoelzel 1991; Guinet and Bouvier 1995). Off the Crozet Islands, intentional stranding behavior is performed by adult females preying upon southern elephant seals (*Mirounga leonina*) (Guinet and Bouvier 1995). Off Punta Norte, Argentina, intentional stranding involves both adult males and females hunting southern elephant seals and southern sea lions (*Otaria flavescens*) (Lopez and Lopez 1985; Hoelzel 1991).

Other odontocete species exhibit considerable intraspecific variability in hunting techniques, group size and social organization. Bottlenose dolphins (*Tursiops truncatus*) exist in nearshore and offshore forms in most parts of their range and have been found to adapt their foraging techniques to a wide range of prey types (Shane *et al.* 1986; Bel'kovich *et al.* 1991).

The results of this study provide further evidence that killer whale populations specialize on particular prey species, especially when successful capture requires highly developed hunting skills and substantial risk to the whales. For example, harbor seal predation in Prince William Sound may require intricate local knowledge of the coastline and location of harbor seal concentrations to efficiently locate prey.

The degree of behavioral flexibility in hunting strategies within local populations of killer whales is unknown. Most studies are carried out during spring, summer, and fall, when killer whales are seen predictably in an area and are feeding on seasonally abundant prey. Little is known of the feeding behavior of the whales when they leave these more easily accessible areas or when winter weather precludes observational research.

The extensive catalogue of documented prey (Hoyt 1984; Jefferson *et al.* 1991; Matkin and Saulitis 1994) suggests that killer whales exhibit behavioral flexibility, as evidenced by the AT1 population's use of two very different foraging strategies to hunt harbor seals and Dall's porpoises. Specializations may be expressed seasonally, or when particular prey species in an area are abundant and reliably encountered. The decline in Steller sea lion and harbor seal numbers in the Gulf of Alaska may result in killer whales using different strategies to exploit alternative species such as sea otters in the Aleutian Islands (Hatfield *et al.* 1998; Estes *et al.* 1998).

The flexiblity in the foraging behavior of killer whales, however, appears to be limited. There is no evidence that transients switch to fish feeding and residents switch to mammal feeding, even seasonally. The differing reactions of potential marine mammal prey species to resident and transient killer whales provide further evidence that feeding preferences for fish and mammals are maintained. The radically different strategies employed in fish-foraging and in mammal-foraging may limit behavioral flexibility and maintain the dietary specializations of residents and transients.

Barrett-Lennard *et al.* (1996) describe profound differences in the characteristics and use of echolocation clicks between residents and transients. The specialized hunting techniques required for salmon feeding, including refinement of echolocation ability and learning of prey avoidance responses, are clearly different than those required for hunting marine mammals. Switching between tactics may also be prohibited by the extent of learning required to efficiently master each hunting technique (Baird *et al.* 1992).

The same factors that promote hunting success for fishes may decrease hunting success for marine mammals. Large group sizes of resident killer whales may actually enhance hunting success for salmon, through the sharing of echolocation information over wide areas (Barrett-Lennard *et al.* 1996), while small group sizes may enhance the hunting success of transients, which depend upon stealth to capture marine mammal prey (Baird and Dill 1996). Passive listening, rather than echolocation, may be employed in the detection of prey by transients (Saulitis 1993; Barrett-Lennard *et al.* 1996).

While distinct fish-eating and mammal-eating populations of killer whales appear to be a common feature in the North Pacific and in other regions such as Antarctica (Berzin and Vladimirov 1983), it can

be expected that populations of killer whales in each area have adapted hunting tactics and dietary specializations that reflect the unique characteristics of their ecosystem.

GIS ANALYSIS

Introduction

Use of a GIS program was used to examine and compare the use patterns of resident and transient killer whales, describe the movement patterns of resident pods and transient groups and determine important areas for these whales. In our area there had been no previous attempt to look at habitat use, however, in other areas differences in habitat use between resident and transient whales have been noted. Heimlich-Boran (1988) noted that resident whales in Georgia Strait, British Columbia, usually traveled from headland to headland and foraged over high relief subsurface topography, while transient whales frequently entered bays and foraged in shallow protected areas, reflecting different strategies for the pursuit of salmon (Oncorhynchus sp.) versus harbor seal (Phoca vitulina richardsi) prey. However, surprisingly little has been published on habitat use of killer whales, even for populations that have been intensively monitored for decades.

Our work focused on thirteen resident pods (approximately 278 individuals), along with at least two assemblages of transient whales, the AT1 and Gulf of Alaska (GOA) transients (approximately 55 individuals at the beginning of the study). The GOA transients are irregular to rare visitors (Matkin et al. 1997). The distribution of killer whale pods in Prince William Sound has been previously discussed by Hall (1986) based on two years of aerial and observations from small vessels (1976 & 1977). An additional thirteen years of at-sea observations and photographic identification were used during this segment of our study (1984-1996). Using these data we tested three hypotheses. First that resident pods and transient groups use different habitats within Prince William Sound. Second, that foraging behaviors were not evenly distributed in these waters and third that resident and transient whales have used these areas less frequently recently than in the past. Documenting the differences between resident and transient habitat use provides a foundation for understanding how the ecology of resident vs. transient whales can drive a differential response to spatially distributed environmental change, including changes in prey (e.g. salmon or pinnipeds) distribution or presence of environmental toxins (e.g. an oil spill).

Methods

The observations reported here are based on identification photographs and behavioral records made between 1984 and 1996, primarily from April to October, over an area of approximately 3500 square kilometers in Prince William Sound, Alaska. Searches were not systematic but relied on observer reports and familiarity with the area and with the behavior of the whales.-For example, in the absence of radio reports from another vessel, searching was located in southwest Prince William Sound (zones 1-3 and 5, Fig. 19, Table 26), while searches in the northern or eastern Sound (zones 6 and 7) and in Hinchinbrook entrance occurred most often in transit from Cordova or Valdez, on receipt of a report from another vessel, or as part of increased search effort in 1989-90 (following the *Exxon Valdez* oil spill). Position for vessel and whale paths were estimated from known landmarks. Within one mile of shore, estimates were likely more accurate (within one hundred meters) than when farther offshore (accurate within ½ to 1 kilometer depending on distance from shore). Whales were approached and individually identified through left-side dorsal fin and saddle patch photography (Bigg et al. 1986, Matkin et al. 1994), and were grouped in pods as defined by Bigg et al. (1990) and Matkin et

al. (1999a). Records, including all vessel and whale paths, were entered into a GIS database for analyses, however, the location of each behavior on the whale track was not noted.

Figure 19. Zones used for analyses of encounter rates. Zone 3 is shaded to make it easier to distinguish. KIP indicates Knight Island Passage.

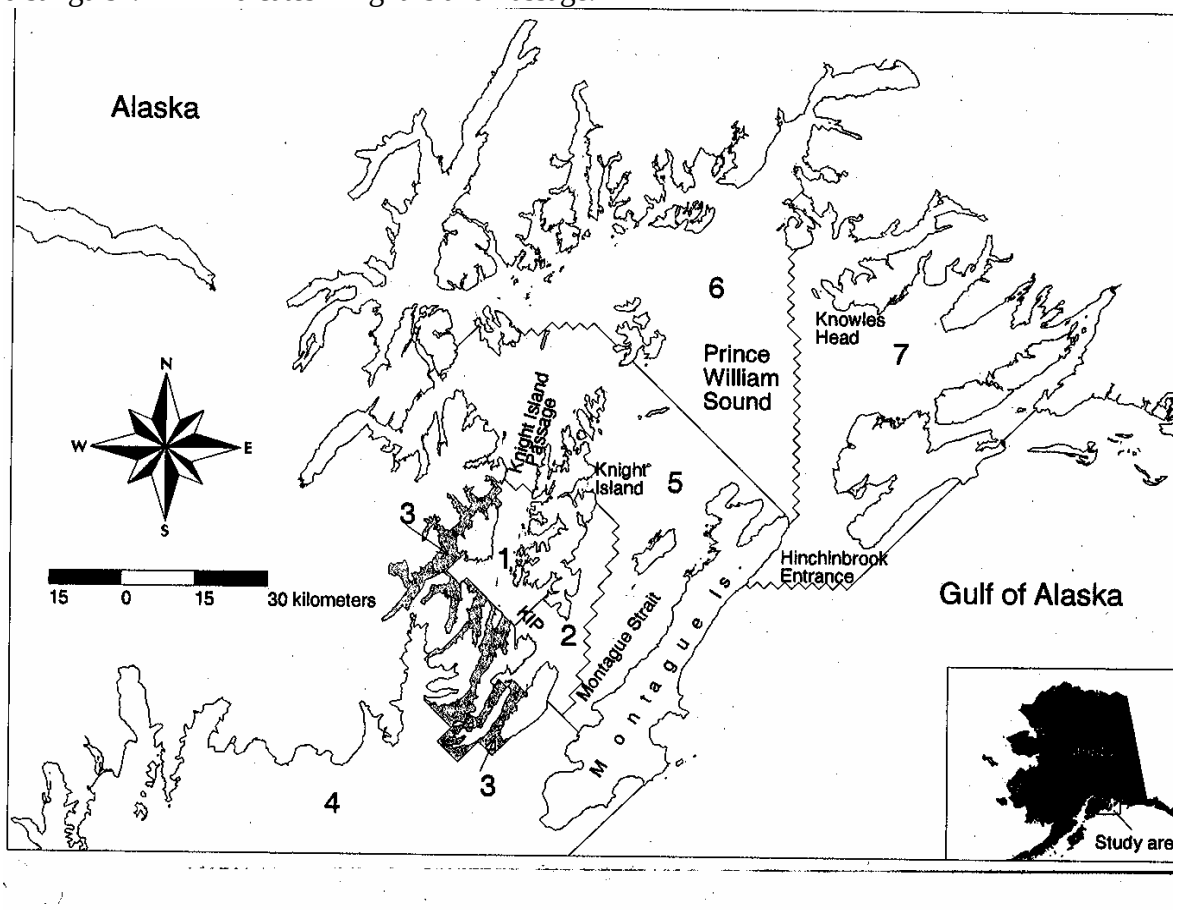


Table 26. (a) Search effort and encounters with killer whale pods by year and (b) search effort by zone over all years.

(a)				(b)	Area		Km searched
Year	Boat-days	Km searched[1]	Encounters[2]	zone	(Km^2)	Km searched[1]	/Km^2
1984	129	11341	69	1	285	30160	105.8
1985	60	4452	48	2	359	28018	78.0

1986	60	4680	34	3	354	8262	23.3
1987	29	2057	22	4	6404	5817	0.9
1988	68	4316	27	5	2270	29878	13.2
1989	206	16181	88	6	3542	11430	3.2
1990	249	19603	85	7	2179	5887	2.7
1991	188	15651	54				
1992	136	10492	69				
1993	79	5591	40				
1994	87	6321	32				
1995	125	11066	63				
1996	92	7700	32				
Total	1508	119452	663			119452	

[1] Kilometers of search effort by all vessels. Note that this statistic and boat days, alternative measures of search effort, are strongly correlated ($R^2 = 0.986$).
[2] Encounters with killer whale groups.

Pod encounter rates corrected for search effort

Search effort was measured as kilometers that each vessel traversed. Kilometers searched and vessel-days were highly correlated (Table 26a), indicating that the average miles searched per day was consistent throughout the study and that either measure of search effort would yield similar results. We divided the study area into seven zones (Fig. 19), based on the distribution of search effort. Areas of dense search effort (e.g. zones 1 and 2, Fig. 19) were divided into small zones; areas of sparse search effort (e.g. zones 4, 6-7) were made into larger zones to increase the sample size of encounters within sparsely searched zones. Zone boundaries were chosen to (i) separate areas of dense and sparse effort (e.g. zone 2 from 5); (ii) keep geographically similar areas in a single zone (e.g. the southwest bays and passages form zone 3, while outside waters form zone 4); and (iii) separate geographically distinct areas (e.g. eastern Sound in zone 7 from northwestern and central Sound in zone 6). Zones were large except in the southwestern Sound (zones 1-3) where search effort was most intense. For each year, we then tabulated the number of encounters with killer whale groups that started within each zone. This number of encounters, divided by the kilometers of effort within that zone, was the encounters-per-unit-

effort. All encounter rates in this paper therefore refer to rates of encounters with known killer whale groups, and not with numbers of individual whales encountered. The composition or size of these groups may have changed over the duration of the study. Encounter rates were an indicator of the frequency of finding whale groups in a particular zone, and were assumed to indicate how commonly groups used different areas of the Sound.

Our analyses of area use consider the AT1 group separately from other transients that also traverse the area and have been collectively known as the Gulf of Alaska (GOA) transients. The separation of these two groups was based on their lack of association as well as genetic separation (Matkin et al 1999b, Barrett-Lennard, in prep.). For resident pods, we limited our analyses to pods with more than 50 encounters over the study period, and thus examined the distribution of the six most frequently encountered resident pods (hereafter referred to as major resident pods): AB, AE, AI, AJ, AK and AN, the latter of which split into two pods, AN10 and AN20, in 1991. For analyses here, we include only sightings of AN10 after the split (there was only a single sighting of the AN20 group in the period 1992-1996). With this exception, all encounters with major resident pods, AT1 transients and the GOA transients are included in our distribution analyses. We calculated encounter rates per unit effort by year and by map zone, and compared the period 1984-1989 (hereafter referred to as the 1980s) with 1990-1996 (referred to as the 1990s). These year groupings were chosen to divide the available data roughly in half, as there were insufficient data to analyze years individually. We evaluated the distribution of social groups across map zone and decades using a multi-variable analysis of covariance (MANCOVA) with Wilk's lambda as the test statistic, and kilometers-of-effort as a covariate to account for search effort. For groups where encounter rates differed significantly by zone, we used a post-hoc Tukey's Honestly Significant Differences multiple comparisons test to identify differences. Tukey's HSD properly accounts for the multiple comparisons being made, so that any further correction (such as the Bonferroni adjustment) is not necessary. In this and subsequent analyses, no statistical difference does not conclusively indicate that differences do not exist, as the data may be too sparse or variable to detect biologically real differences.

Foraging behavior

Whales were followed and their behavior recorded as opportunity permitted. Behaviors were classified as Travel (movement on a consistent compass course; group members surfaced and dove synchronously), Rest (slower than normal movement; maternal units were in close association (<1 body length from neighbors) and synchronous in movement and breathing), Social (interaction between individuals, including sexual behaviors, chasing, rolling; breaching, spy-hopping, fluke and flipper slapping), or Foraging (any activity related to search for, pursuit of, capture and consumption of prey). Foraging was broken down into sub-behaviors: Feeding (prey seen in the mouth of a whale or surface indications of prey such as blood, oily sheen, or fish scales), and for resident pods, Foraging for Fish (tight circling, rapid erratic movement, and lunges often accompanied by echolocation), or for transient whales, Open water Foraging for Mammals (milling or slow travel when at the surface $\geq$ 1 km offshore, silent dives of ten or more minutes duration and underwater movements of $\geq$ 1 km between surfacing) and Nearshore Foraging (movement following contours of the shoreline often within 20 m of shore, and entering small bays, narrow channels, and exploring rock outcrops or shoal areas).

Foraging sub-behaviors were not reliably distinguished in the field before 1987, and we therefore restricted analyses of behavior to 1987 and later years. Although the path of each encounter and the duration of behaviors were recorded, the specific locations of different behaviors were not. The

distance traveled by whales was tabulated from the GIS database, and the zone in which most of this distance (>50%) occurred was designated the "behavior zone" for that encounter (to distinguish this special use from any zone that contained a portion of the encounter. Encounters where no single zone contained >50% of the path length were designated as behavior zone 9, a separate classification). Behaviors were analyzed based on behavior zone and for the periods 1987-1991 and 1992-1996 (It was necessary to combine years because of small sample sizes. The sampling period for behavioral data was shorter than for distributional data, and the time periods were chosen simply to divide the available data roughly in half.). We calculated the proportion of each encounter duration that was spent in Foraging activities other than Feeding. We did not include Feeding because we were interested in the choices whales make about where to acquire prey, rather than in whether habitat has an effect on the time taken to consume prey already obtained. For the same reason, we analyzed behaviors as the time-proportion of encounters (each encounter weighted equally) rather than analyzing time-weighted behaviors (a time budget). Thus, each observation bout (encounter) represents an independent sample of where whales were found foraging, and we avoid the problems of non-independence of sequential behaviors and of potential bias in encounter length by geographic location. Proportions were arc-sin transformed and their distribution analyzed by time period and behavior zone using ANOVAs on the arc-sin transformed proportions. Major resident pods that were similar in their area use patterns (see Distribution Results, Table 28), were combined for analyses of behavior (AB, AI, AN and AJ were considered together, as were AE and AK). GOA transient whales were excluded from analyses of behavior because foraging observations for these groups were too sparse for meaningful analyses.

Results

Surveys resulted in a total of 1508 boat-days of search effort and 663 encounters with 19 different killer whale groups over thirteen years. The most intense searching was conducted in western Montague Strait and Knight Island Passage. These areas were designated as zone 1 (Fig. 1; encompassing south western Knight Island Passage, immediately around southwestern Knight Island where researchers maintained a base camp during most years of study) and as zone 2 (Fig. 1; including eastern Knight Island Passage and western portions of Montague Strait, the remainder of the most intensely searched area). The outer, central, and eastern areas of the Sound (zones 4, 6 and 7, respectively) received relatively sparse coverage. The remaining two zones, zone 5 around northern and eastern Knight Island complex, and zone 3 in the southwest bays and passages, received intermediate levels of effort . Encounters involving the six major resident pods, the AT1 group or any of the GOA transient groups made up 96% (N = 638 encounters) of all encounters (Table 27).

Table 27. The number of encounters in which each pod or group was seen, 1984 to 1996 (N = 638 encounters, some of which contained multiple pods).

Pod	N
AT1	160
GOA	24
All residents	461
AB	220
AE	145

AI	168
AJ	56
AK	89
AN	147

Table 28. Results of a MANCOVA showing overall and univariate effects of zone and decade on encounters with six resident pods and AT1 and GOA transient groups (see text for details).

Analysis	source	df	Approx. F	p ≤	Effect[1] (HSD)	
MANCOVA	Effort[2]	8, 75	22.67	0.001	+	
	Zone	48, 373	2.21	0.001	see univariate tests	
	Decade	8, 75	2.98	0.006	see univariate tests	
AB (Resident)	Zone	6	5.68	0.001	2 1 7 4 5 3 6	(3.27)
	Decade	1	1.77	0.187	NS	
AI (Resident)	Zone	6	5.86	0.001	2 1 7 4 5 3 6	(2.38)
	Decade	1	0.50	0.482	NS	
AJ (Resident)	Zone	6	2.41	0.034	2 1 4 3 7 6 5	(1.39)
	Decade	1	1.39	0.242	NS	
AN (Resident)	Zone	6	5.14	0.001	2 1 4 7 3 6 5	(2.80)
	Decade	1	0	0.985	NS	
AE (Resident)	Zone	6	4.01	0.001	1 2 5 7 4 6 3	(1.73)
	Decade	1	4.15	0.045	90s > 80s*	
AK (Resident)	Zone	6	1.45	0.205	NS	
	Decade	1	0	0.994	NS	
AT1 (Transient)	Zone	6	4.16	0.001	1 3 7 6 4 5 2	(2.17)
	Decade	1	9.36	0.003	80s > 90s*	
GOA (Transient)	Zone	6	3.22	0.007	5 3 4 6 7 2 1	(0.87)
	Decade	1	1.15	0.286	NS	

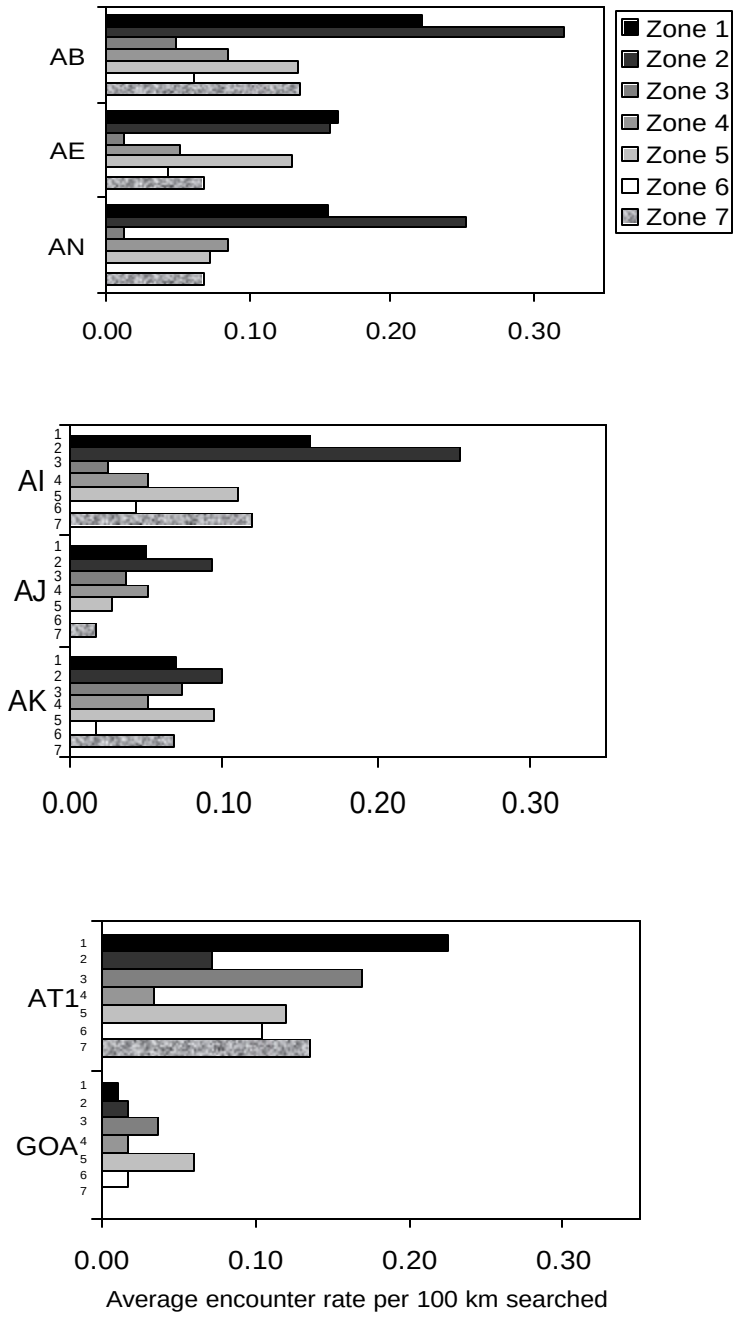
[1] Plus indicates that effort was positively correlated with encounter rates. Zone numbers appear ordered from most to least encounters (after effects of effort and decade have been accounted for); bars connect zones that were not significantly different (Tukey's Honestly Significant Differences multiple comparisons test, HSD in parentheses).
[2]The effect of effort in each univariate comparison was significant ($F \geq 5.52$, $df = 1$, $p \leq 0.021$) however the individual statistics were not listed to save space.

Distribution

Over all major resident pods and transient groups, encounters increased with search effort and were affected by zone and decade. Average encounter rates varied greatly year to year with no significant differences between decades, except that encounter rates were generally higher in the 1980s than the 1990s for AT1 group, and higher in the 1990s than 1980s for AE pod (Table 28, univariate results). For AT1 group, encounter rates during the 1980s were less than 0.1 per 100 km searched in 1984, but were more than twice that in 1985, 1988, and 1989. In contrast, encounter rates for this group were below 0.1 per 100 km searched in 1991, 1994, and 1996; and did not rise above 0.2 per 100 km searched at any time during the 1990s. For AE pod in the 1980s, there were only two years when encounter rates were higher than 0.1 per 100 km searched (1985 and 1986), while encounters were at least that high in six of seven years in the 1990s.

With the exception of AK pod, all major resident pods tended to use Knight Island Passage (zones 1-2) more than other areas of the Sound (Figure 20). Area use patterns were especially similar for resident pods AB, AI, and AN. Collectively, these three pods were recorded in south west bays and passages (zone 3) only 14 times (4.6% of 303 encounters), only once (0.3% of encounters) in the northwestern Sound, eight times (2.6%) in the central Sound, once (0.3%) in the eastern Sound and twelve times (4.0%) in the area of Hinchinbrook Entrance. All other encounters were to the southwestern Sound, primarily Knight Island Passage and Montague Strait (zones 1, 2 and 5). Among major resident pods, the pattern exhibited by these three pods was most different from that of pod AK, which showed no statistical preference for any of the zones (Fig. 20). Of the two remaining major resident pods, AE was more similar to the AK pattern of no strong preference for a particular portion of the study area. For AE pod, slightly higher use in the most-frequented zones (1 & 2) was not significantly different from use in the least-used zone (zone 3) in pair-wise comparisons using Tukey's Honestly Significant Difference. Pair-wise comparisons, as in this case, may fail to reject a null hypothesis even when the overall statistic can reject it based on the larger overall sample size). Finally, the pattern shown by AJ pod was similar to but less dramatic than that of pods AB, AI and AN. Area use by AJ pod was greatest in zones 1 & 2, and the most-frequented and least-frequented zones were significantly different (Table 28). Although all six major resident pods used zone 3 in transit, none showed a pattern of regular occurrence in these passages or the southwestern bays.

Figure 20. Encounter rates with major resident pods and the AT1 and GOA transient groups in zones 1-7. Numbers for each bar indicate the zone for which encounter rates are shown.



These patterns of area use by the major resident pods differed from those of the AT1 transient group or the combined GOA transient groups, which used a larger portion of Prince William Sound than resident pods AB, AI, AN, or AJ and were more likely to be encountered in zone 3. The AT1 group also used the mid- and eastern-Sound waters (zones 6 and 7, Figure 21) more commonly than any

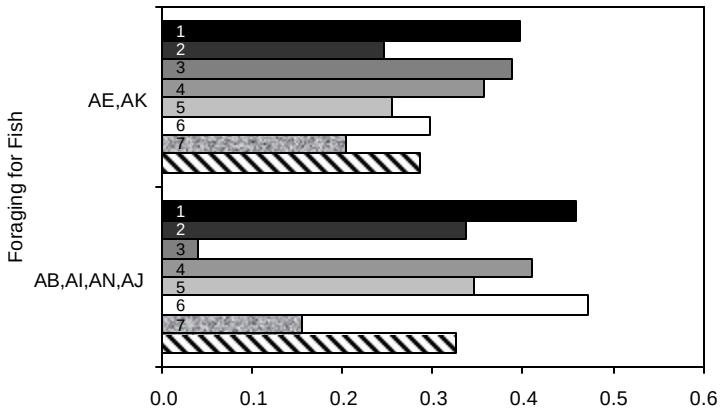
other group. The GOA transients used areas almost exclusively in southern or eastern Montague Strait (zones 4 and 5., the north tip of Montague Island (zone 5), and in Hinchinbrook entrance.

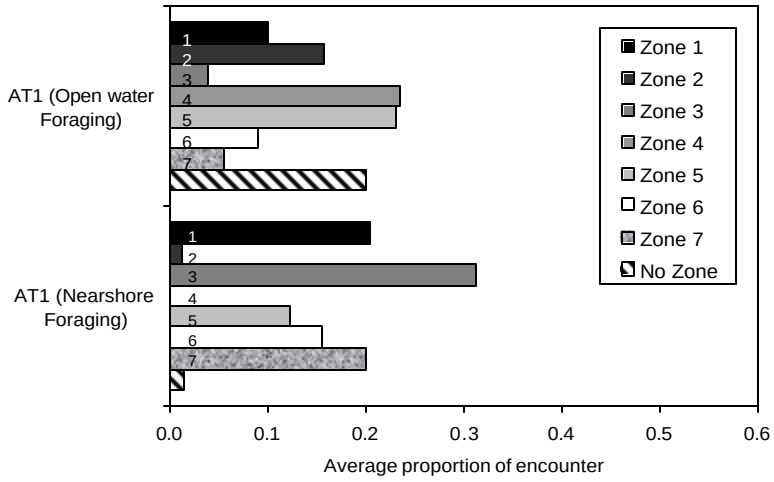
Distribution of foraging behaviors

Foraging for Fish made up a greater proportion of sample time with AB, AI, AN and AJ pods in the period 1987-1991 than in the period 1992-1996, but there were no significant differences between behavior zones in the incidence of this foraging behavior (Fig. 3. ANOVA: time period, df = 1, F = 5.41, p = 0.021; behavior zone, df = 7, F = 0.44, p = 0.876). The same pattern was found for pods AE and AK considered together: the occurrence of Foraging for Fish was greater for the 1987-1991 time period than during the 1992-1996 period, but there were no significant differences among behavior zones (Fig.21 ANOVA: time period, df = 1, F = 4.17, p = 0.042; behavior zone, df = 7, F = 1.17, p = 0.318).

In contrast, for the AT1 group the occurrence of Nearshore Foraging was significantly greater on encounters that occurred predominantly in zones 1 and 3 and less on encounters predominantly in zones 2 and 5 (Fig. 21 ANOVA: behavior zone, df = 7, F = 2.16, p = 0.043). No differences were found for this behavior by time period (ANOVA: df = 1, F = 0.18, p = 0.670). There were no significant differences in the occurrence of Open water Foraging for Mammals across behavior zones or time periods for this group (ANOVA: time period, df = 1, F = 0.01, p = 0.923; behavior zone, df = 7, F = 0.65, p = 0.710).

Figure 21. (top panel) Incidence of Foraging for Fish behavior by zone in which greater than 50% of encounter occurred, and (bottom panel) incidence of Transient Nearshore Foraging and Open-water Foraging for Mammals by the zone in which greater than 50% of encounter occurred (see Methods for details). Numbers for each bar indicate behavior zone for which behavior rates are shown.





Discussion

Most individual killer whales from pods considered in this study are known only from Prince William Sound and surrounding waters. The Sound is a relatively small area, 200 km (90 miles) at its widest, and resident killer whales are known to have ranges in excess of 750 km (Biggs et al. 1990, Matkin et al. 1997). It is therefore not surprising that every group examined was recorded at least occasionally throughout Prince William Sound as well as to the west of the Sound in the waters off the Kenai Peninsula.

The frequency of encounters with most major resident pods did not differ significantly between the 1980s (1984-1989) and the 1990s (1990-1996). Differences were found only for the AE pod, which was more frequently encountered in the 1990s. However, these analyses examined only the frequency of encounters with the major resident pods, and did not consider the number of individual whales present at each encounter nor the presence of other less well known resident pods that entered the Sound on occasion and traveled with the major resident pods. In particular, the decline in the size of AB pod from 36 to 22 individuals following the *Exxon Valdez* oil spill (Matkin et al. 1999a) was not considered. Also, in 1990 AN pod split into AN10 and AN20 pods and the 28 whales in the AN20 pod were no longer encountered in the Sound. Our results indicate that the six major resident pods continued to use the Sound consistently over the years, even while that the number of resident whales present in some pods declined (Matkin et al. 1999a). In addition, the number of other resident pods using the Sound may have declined and resulted in an overall decline in use of Prince William Sound by resident killer whales (Matkin et al 1999c).

The transient AT1 group frequented the Sound less often in the 1990s than in the 1980s. These animals seldom traveled as a single unit and were generally encountered in groups of 2 to 6 whales. The total number of AT1 whales declined from 22 to 11 whales during the early 1990s following the *Exxon Valdez* oil spill. (Matkin et al. 1999b,c). In addition, harbor seals, the primary prey of the AT1 group, have also declined during this period (see discussion of behavioral data, below) and these killer whales may have been forced to increase their foraging range in response to the decrease in harbor seal numbers.

From the onset of the study in 1984, the primary focus of this research has been photoidentification. Data on distribution of killer whales was not collected in a systematic format designed to answer specific questions regarding changes in distribution. Thus, the uses of the data to

examine distribution are limited and must be approached with caution. However, biases are minimized when data are stratified by search effort (as here) or by sighting density (Edwards & Kleiber 1989). Spatial biases may occur if a unit of search effort in one area of the Sound is not as efficient as the same effort in another area. Because researchers concentrated search effort in areas where they were most successful at sighting whales, it seems likely that any spatial bias would be towards underestimating use in areas where search effort was lowest (zones 4, 6 and 7.). However, we note that sightings per unit effort in these zones do not appear unusually low. Temporal biases may occur if observers over time became more skilled and more familiar with the areas used by whales. Temporal biases are likely to favor an increase in sightings per unit effort over time, a trend found for AE pod but opposite to that detected for the AT1. With these caveats in mind, the direction of potential bias allows us more confidence of the effect in the AT1 group but perhaps less so for AE pod.

We found several general patterns of spatial use among eight different resident pods and transient groups. A major difference was apparent between resident and transient-type whales. The partitioning of habitat between residents, which occurred in the wider entry waterways of the western Sound (Montague Strait and Knight Island Passage), and transients, which were more often found in the narrow bays and passages (zone 3), reflects dietary preferences. Salmon migration pathways enter the Sound at Montague Strait and run up passages along the western side of Knight Island, and resident whales feed on the salmon across the width of these channels (Saulitis et al. 2000). However, foraging tactics on pinnipeds appear to require careful searching of areas very close to shorelines (Saulitis et al. 2000), perhaps because pinnipeds are most vulnerable as they enter or leave haul-out sites. Data on the distributions of salmon, pinniped, or cetacean prey within the Sound have yet to be published.

In British Columbia, transient whales exhibited group-specific foraging behavior: some transient groups seldom foraged in nearshore areas while others spent up to 50% of their time foraging nearshore (Baird & Dill 1995). Members of the AT1 group spent similar amounts of time in nearshore and offshore foraging (Fig. 21). Our analyses were consistent with the interpretation that killer whale distributions reflect their foraging needs, at least in some cases. For example, Nearshore Foraging for the AT1 group (Fig. 21) was significantly more common in the southwest bays and passages (zone 3), along the western side of Knight Island (zone 1) and in zone 7, the same areas where this group spends a disproportionate amount of its time (Fig. 20) suggesting these areas were critical foraging habitat for these whales. However, no similar pattern emerged for Open water Foraging for Mammals behavior for the AT1 group. For the resident groups, foraging behaviors were no more likely in the most commonly used zones than they were elsewhere. Although we found a decrease from the 80s to the 90s in the proportion of time major resident pods in the Sound spent foraging, there was no evidence that foraging behavior accounts for differences between the AB, AN, AI and AJ grouping of resident pods and the AE and AK grouping. It should be noted that although members of all these pods intermix at times, the AB, AN, AI and AJ pods are genetically distinct (Mt DNA) from the AE and AK pods (Matkin et. al. 1999b, Barrett-Lennard et. al in prep).

Harbor seal numbers in Prince William Sound have declined by 63% from 1984 through 1997, a decrease that was exacerbated by the *Exxon Valdez* oil spill (Frost et al. 1999). Harbor seals are the primary prey of the AT1 transient group (Saulitis et al. 2000). It is possible that a decline in prey availability accounts for the general decline in encounter rates with the AT1 group. These whales appeared to use the Sound heavily for foraging, as the frequency of Nearshore Foraging is highest for AT1 whales in the areas where they most often occur. Nearshore Foraging is exhibited when whales are hunting pinniped prey, primarily harbor seals (Saulitis et al. 2000).

Killer whale distributions may also depend on the locations of favored rubbing beaches or other resources, although there is no evidence to support or refute such a claim. Differences in foraging behavior or in the distribution of prey seem likely to account for area use differences between residents and transients. In British Columbia, two genetically distinct, non-associating resident communities exist whose ranges seldom overlap (Bigg et al. 1990, Ford et al. 2000) and it may be that distinct ranges resulted from competition and the specialization of each community on different salmon runs. Although we found two different patterns of area use among genetically distinct, occasionally associating groups of resident whales in the Sound, these patterns overlap strongly and are apparently not similar to the geographic distinction between communities in B.C. We found no evidence from analyses of foraging behavior that would account for the differences in area use the among resident pods in the Sound that we examined.

In light of increased discussion of marine reserves and habitat protection, this study is a first attempt to define specific marine habitats important for wide ranging Odontoceti such as killer whales.

Although killer whale distribution in relation to salmon abundance has been examined in Puget Sound (Heimlich-Boran 1986), there has been no attempt in other areas of the North Pacific to define important killer whale habitat. In Prince William Sound, killer whales are predictably found inshore, at least seasonally, as are their prey. Such an approach may have application to other Odontoceti where data are available.

CONTAMINANTS

Introduction

Organochlorines (OCs) are persistent chemical contaminants that frequently occur in the marine environment. Many of these compounds, including chlorobiphenyls (CBs) and DDTs, are highly lipophilic and can bioaccumulate in relatively high concentrations in top level predators of the marine food web through trophic transfer. Because many of these contaminants are toxic to humans and wildlife, open uses and the manufacture of CBs in the U.S. was ceased in 1977 (Beeton et al. 1979) and use of DDT was banned for use in the U. S. in 1972 (Ahmed 1991). However, several of these compounds continue to be used as agricultural and industrial chemicals in other parts of the world, including countries from South America and Asia (Schmidt, 1998). OCs enter the marine environment via several sources (i.e., atmospheric transport, landfill runoff) and are found in environmental samples from all over the world, including remote, non-industrial areas such as Alaska, the Canadian Arctic, Greenland (AMAP, 1998; Barrie et al., 1992; Iwata et al., 1993; Muir et al., 1992).

Killer whales (Orcinus orca), the largest species in the Delphinidae family, are relatively long-lived animals with mean life expectancies of approximately 50 years for females and 30 years for males (Olesiuk et al., 1990), and maximum life expectancies of 80 - 90 years for females and 50 - 60 years for males. These animals are abundant in coastal waters and high latitudes, with well-studied populations occurring in Puget Sound, the inside waters of British Columbia, Southeastern Alaska and Kenai Fjords/Prince William Sound, Alaska. Two eco-types of killer whales, "transient" and "resident", occur in all of these regions (Bigg, 1982; Bigg et al., 1990; Dahlheim, 1997; Matkin et al., 1999a). These eco-types are genetically distinct (Hoelzel et al., 1998; Barrett-Lennard, pers. comm.) and differ in various aspects of morphology, vocalization patterns and habitat use (Bigg, 1987; Morton, 1990; Jurk, pers. comm.). The social structure of resident killer whale populations from the Eastern North Pacific appears complex. The residents travel in large groups called pods, which center on mature females and are considered matriarchal societies. The resident killer whales in the Kenai Fjords/Prince William Sound region travel in stable pods of 6 - 36 individuals composed of females and their descendents (Matkin et al., 1999a,b). Pod membership is supported by pod specific vocal dialect (Bigg et al., 1990; Ford, 1991). In contrast, transient whales from this region travel in smaller, more fluid groups than residents (typically composed of 1 – 7 individuals) (Matkin et al., 1999b). Although transient groups may consist of a female whale and her offspring, some transient groups are composed of only males (Matkin et al., 1999b). Genetic and photo-identification studies of Alaskan killer whales have provided information on the male – female composition of most of these resident pods and transients groups, as well as the approximate ages, reproductive status and putative recruitment order of the individual whales (Heise et al., 1992; Dahlheim, 1997; Hoelzel et al., 1998; Matkin et al., 1999a,b).

The resident and transient killer whales from the Kenai Fjords/Prince William Sound, AK region have distinct dietary preferences and feed at different trophic levels. Saulitis et al. (2000) found that

Prince William Sound transients feed on marine mammals, primarily harbor seals and Dall's porpoise while the sympatric resident whales eat fish, primarily salmon. Transients often feed along shorelines and in glacial areas while residents most often forage offshore (Sheel, pers. comm.). Because of differences in diet and habitat use by resident and transient killer whales, differing contaminant levels might be expected in the two killer whale ecotypes. Relatively high levels of contaminants might be expected in killer whales, especially transients, since they are top level predators in Eastern North Pacific waters.

Little comprehensive contaminant data are available for Alaskan killer whales, especially free-ranging animals. As part of a collaborative study between the Environmental Conservation Division at the Northwest Fisheries Science Center and the North Gulf Oceanic Society, blubber samples were acquired by biopsy from free-ranging killer whales in the Prince William Sound/Kenai Fjords region from 1994 - 1999 to determine if killer whales have levels of toxic OC contaminants that could negatively affect the whales. From these data, the influences of diet as well as biological factors including sex, reproductive status and recruiment (birth) order on contamination concentrations were assessed.

Methods

Whale Identification

Identification photographs were taken of the individual at the time of sampling to confirm its identity or for later identification using the method of Bigg et al. (1986) and is explained in detail in the "Field Methods" section of this report. For each biopsied killer whale we genetically determined eco-type (resident or transient) and pod or group membership. Information on sex, approximate age (e.g., < 34, 28?), reproductive status (i.e., sexually immature, reproductive female, sexually mature male) and putative recruitment order (i.e., first-recruited, non-first-recruited) were determined using methods detailed in Matkin et al. (1999b). Reproductive status of each killer whale was based on age, association analysis and direct observation (Matkin et al., 1999a,b). Killer whales < 15 years of age were classified as sexually immature (except AI04, a female known to have given birth at age 13), females = 15 years of age were grouped as reproductive and males = 15 years of age were designated as sexually mature. Putative recruitment order of certain resident killer whales was inferred by direct observation (Matkin et al., 1999a,b).

Field Sample Collection

Biopsy samples for chemical contaminant analyses were collected from 77 individual free-ranging killer whales from the Kenai Fjords/Prince William Sound region during the months May - September, 1994 – 1999. The blubber portion of the biopsy sample was excised using a solvent-rinsed scalpel, placed in a solvent rinsed glass vial and stored at –20°C until chemical analysis as described in the "Field methods" section of this report.

Analytical techniques

Biopsy blubber samples of killer whales were analyzed by a high-performance liquid chromatography/photodiode array (HPLC/PDA) method (Krahn et al., 1994) that was developed to rapidly measure concentrations of dioxin-like CBs and other selected OCs in various tissues of commercially and recreationally important marine species (Ylitalo et al., 1999). Briefly, blubber (0.1-0.4 g), hexane/pentane (1:1 v/v), sodium sulfate (5 g) and a surrogate standard (1,7,8-trichlorodibenzo-p-dioxin; 250 ng) were homogenized for 2 minutes. The sample mixture was then centrifuged and the extract was decanted into a 50-mL concentrator tube. The homogenization step was repeated and the extracts were combined. A 1-mL aliquot of sample extract was removed for lipid analyses and the

remaining sample extract was reduced in volume to ~ 1 mL. The sample extract was loaded onto a gravity-flow cleanup column, comprised of a glass wool plug, silica gel, basic silica gel and acidic silica gel, to separate the desired analytes from other interfering compounds (e.g., lipids, aromatic compounds). The analytes were eluted from the cleanup column with 14 mL hexane/methylene chloride (1:1 v/v) and collected into a clean 50-mL concentrator tube. The HPLC internal standard (1,2,3,4-tetrachlorodibenzo-p-dioxin; 250 ng) was added to each sample and the solvent volume was reduced by nitrogen to ~ 150 µL.

Eight dioxin-like congeners (PCBs 77, 105, 118, 126, 156, 157, 169, 189) were resolved from other selected CBs (PCBs 101, 128, 138, 153, 170, 180) and chlorinated hydrocarbons (o,p'-DDD, p,p'-DDD, p,p'-DDE, o,p'-DDT, p,p'-DDT) by HPLC on two Cosmosil PYE analytical columns, connected in series and cooled to 16°C. The congeners were measured by an ultraviolet (UV) photodiode array detector and were identified by comparing their UV spectra (200-310 nm) and retention times to those of reference standards in a library. The analyte purity was confirmed by comparing spectra within a peak to the apex spectrum. In some cases, certain CB congeners coelute with other CBs with the HPLC/PDA method. For example, CB 101 coelutes with CBs 99/149/196 and possibly with others, CB 153 coelutes with CB87 and CB 170 coelutes with CB194.

The HPLC system was calibrated daily. A sample set consisted of 11 – 14 field samples, a method blank and quality assurance samples. Method blanks contained no more than five analytes that exceeded four times the method detection limit (MDL), unless the analyte was not detected in the associated blubber samples of the set. Approximately 10% of the whale blubber samples were analyzed in duplicate to measure precision of the method and the laboratory quality assurance criteria were met for all analytes detected in the blubber samples. To monitor the accuracy of our HPLC/PDA method, a National Institute of Standards and Technology (NIST) control whale blubber sample was analyzed with each sample set and results met laboratory criteria (Wise et al., 1993). The limits of detection (LOD) for the CB congeners ranged from < 0.46 to < 14 ng/g, wet weight. The LOD for the DDTs ranged from < 2.5 to < 17 ng/g.

The mass of each biopsy blubber sample was small (less than 0.50 g), therefore the entire sample was used for OC analyses. In order to determine lipid content of each sample, a 1-mL aliquot of each sample extract was set aside for lipid analysis using thin layer chromatography coupled with flame ionization detection (TLC/FID) (Shantha, 1992). Each lipid sample extract was spotted on a Chromarod (Type SIII) and developed in a solvent system containing 60:10:0.02 hexane:diethyl ether:formic acid (v/v/v). Various classes of lipids (i.e., wax esters, triglycerides, free fatty acids, cholesterol and polar lipids) were separated based on polarity, with the nonpolar compounds (i.e., wax esters) eluting first, followed by the more polar lipids (i.e., phospholipids). The lipid concentrations were determined using an Iatroscan Mark 5 (Iatron Laboratories, Tokyo, Japan), operated with a hydrogen flow rate of 160 ml/min and air flow of 2000 ml/min. Data were acquired and analyzed on a 386 PC compatible computer using TDatascan software (RSS Inc., Bemis, TN). A four-point linear external calibration was used for quantitation. Total lipid concentrations were calculated by adding the concentrations of the five lipid classes for each sample and were reported as percent total lipid. Duplicate TLC/FID analyses were performed for each sample extract and the mean value reported.

Calculated values

Total CB (?CB) concentrations were calculated using the following formula: ?CBs = ? concentrations of selected CBs (based on individual response factor) + ? concentrations of other CB congeners (calculated by summing areas of peaks identified as CBs and using an average CB response factor). Summed DDT (? DDTs) concentrations were calculated by adding the concentrations of five

DDTs (o,p'-DDD, p,p'-DDD, p,p'-DDE, o,p'-DDT, p,p'-DDT) determined by our HPLC/PDA method. Summed DDT and total CB concentrations were reported as ng/g, wet weight or lipid weight.

To assess the toxic potency of the dioxin-like CBs in the whale blubber samples, CB TEQs were calculated according to the method of Safe (1990) using an additive model of toxicity. In this method, the molar concentration of each dioxin-like CB congener was multiplied by the appropriate toxic equivalency factor (TEF), recommended recently by World Health Organization for human and wildlife health (Van den Berg et al., 1998). The following TEF values, which are based on several in vivo and in vitro studies, including human, mammalian and avian investigations, were used for TEQ calculations: CB77 (0.0001), CB105 (0.0001), CB118 (0.0001), CB126 (0.1), CB156 (0.0005), CB157 (0.0005), CB169 (0.01) and CB189 (0.0001). The CB TEQs are reported as pg/g, wet weight or lipid weight.

The TEQs calculated for the blubber of killer whales in the current study are conservative values. For example, only concentrations of dioxin-like CBs are determined by our HPLC/PDA method while concentrations of other dioxin-like compounds, such as polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), are not. As a result, the TEQs calculated for the killer whale samples are based solely on dioxin-like CBs and do not include PCDDs and PCDFs that, if detected, would increase the TEQ values of the biopsy samples. However, several marine mammal contaminant studies have shown that PCDDs and PCDFs contribute much less (usually < than 15%) to the TEQ values compared to the dioxin-like CBs in cetaceans from various parts of the world (Kannan et al., 1989; Jarman et al., 1996; Addison et al., 1999; Jimenez et al., 2000; Ross et al., 2000). This is because dioxin-like CBs are often found in much higher concentrations than are the polychlorinated dibenzodioxins (PCDDs) or dibenzofurans (PCDFs) in the marine environment even though dioxin-like CBs are only 0.00001 to 0.1 times as toxic as TCDD (Van den Berg et al., 1998). Furthermore, our HPLC/PDA method has higher limits of detection (LODs) for certain congeners, especially the non-ortho-substituted CBs (e.g., CBs 77, 126, 169) compared to the LODs of more comprehensive analytical methods (i.e., high-resolution gas chromatography/mass spectrometry). In addition, irrespective of analytical method, the small masses (< 0.5 g) of the killer whale biopsy samples also contribute to higher LOD for certain mono-ortho- and non-ortho-substituted congeners. Consequently, the TEQs determined in the killer whale biopsy samples were conservative values because sample size is small, and they did not include PCDDs, PCDFs or any dioxin-like congeners (e.g., CBs 77, 126, 169) that were below the LOD in the TEQ calculation.

Statistical analyses

Lipid concentrations were arcsine square root transformed and OC concentrations were log transformed to increase the homogeneity of variance. Stepwise regression analysis was used to evaluate relationships among OC exposure (e.g., CB congeners, DDT, DDT metabolites, ?CBs, ?CB TEQs) and life-history parameters (e.g., age, sex, birth order; see Table 1) in killer whales to determine the life-history parameters (e.g., eco-type, sex, reproductive status, birth order) that most closely correlated to OC concentrations. Analysis of variance (ANOVA) and the Tukey-Kramer honestly significant difference test (HSD) were used to compare mean concentrations of OCs between transient and resident whales. For resident whales, analysis of variance and the Tukey-Kramer HSD test were used to determine differences in mean concentrations of OCs among three reproductive groups [sexually immature whales (both males and females), reproductive females, sexually mature males]. The level of significance used for all statistical tests was $p = 0.05$. All statistical analyses were completed using JMP Statistical Software (SAS Institute, Inc., Cary, NC).

Results

Although wide ranges of OC levels were measured in the killer whale biopsy samples, significantly higher OC concentrations were measured in blubber of transient killer whales compared to the levels found in the blubber of residents based on both wet weight and lipid weight values (Tables 29 and 30). For example, the mean ? DDTs concentration in transient whales was approximately 25 times as great as the mean level in resident whales. Similar results were observed for ?CBs, with transient whales containing a mean ?CB concentration more than 15 times the mean level in residents.

The most abundant OC analyte (Table 28) measured in blubber of Alaskan killer whales was the DDT metabolite, p,p'-DDE, with concentrations (wet weight) ranging in resident whales from 150 - 22,000 ng/g and 21,000 – 210,000 ng/g in transients. This DDT metabolite accounted for approximately 80% of ? DDTs measured in resident whales and 86% of ? DDTs in the transient whales.

The moderately chlorinated ortho-substituted congeners (i.e., CBs 138, 153) were the predominant CBs measured in the killer whale blubber (Table 29). Similar to the ?CB and ?DDT concentration data, we also found much higher concentrations of individual CBs in transient whale blubber compared to those in the residents. For example, CB 118 concentrations (based on lipid weight) ranged from 60 – 3,400 ng/g in residents and 1,400 – 18,000 ng/g in transients. Dioxin-like CBs (e.g., CBs 105, 118, 156, 157, 189) were also determined in whale blubber samples, with the mono-ortho-substituted congeners being most abundant. In addition, a greater number of dioxin-like congeners were measured in the transient whales compared to the number of these congeners found in the residents. The two mono-ortho-substituted dioxin-like congeners (CBs 157 and 189) were measured in 60% of the transient whale samples but were detected in less than 20% of the resident samples. The most toxic CB congeners, the non-ortho-substituted congeners, CBs 77, 126 and 169, were not detected in any of the tissue samples analyzed, with the LOD ranging from 0.46 – 14 ng/g, wet weight.

Table 29. Mean concentrations (X± SD ng/g, wet weight or ng/g, lipid weight of dioxin-like CBs and other selected CBs in biopsy blubber or resident and transient killer whales from the Kenai Fjords/Prince William Sound, AK region

	Dioxin-like CB congeners[a]				
Whale Form	***105****	***118****	***156****	***157***	***189****
Resident (n = 64)	50 ± 54e	200 ± 220	14 ± 14b	NR	NR

(ng/g, wet wt.)	(ND - 230)	(13 - 940)	(ND - 60)			
Resident (n = 64)	170 ± 160e	710 ± 700	49 ± 36b	NR	NR	
(ng/g, lipid wt.)	(ND - 850)	(60 - 3,400)	(ND - 140)			
Transient (n = 13)	320 ± 290	1,600 ± 1,400	54 ± 31	I[d]	17 ± 12a	
(ng/g, wet wt.)	(19 - 890)	(100 - 4,400)	(4.3 - 120)		(ND - 39)	
Transient (n = 13)	1,200 ± 970	6,200 ± 4,900	220 ± 100	I[d]	76 ± 40a	
(ng/g, lipid wt.)	(260 - 3,700)	(1,400 - 18,000)	(58 - 430)		(ND - 160)	

	Other selected CB congeners					
Whale Form	***101£****	***128****	***138****	***153/87****	***170/194****	***180****
Resident (n = 64)	620 ± 620f	66 ± 68d	310 ± 370	660 ± 680f	70 ± 74c	170 ± 200
(ng/g, wet wt.)	(ND - 2,900)	(ND - 300)	(16 - 1,700)	(ND - 2,900)	(ND - 340)	(12 - 1,000)
Resident (n = 64)	2,200 ± 2,100f	270 ± 310d	1,100 ± 1,100	2,300 ± 2,100f	250 ± 210c	610 ± 540
(ng/g, lipid wt.)	(ND - 11,000)	(ND - 2,100)	(64 - 4,500)	(ND - 9,000)	(ND - 820)	(44 - 2,500)
Transient (n = 13)	8,600 ± 6,100	1,300 ± 820	5,300 ± 3,500	9,900 ± 6,500	1,100 ± 560	2,900 ± 1,600
(ng/g, wet wt.)	(870 - 22,000)	(120 - 2,600)	(460 - 12,000)	(1,100 - 24,000)	(110 - 2,000)	(240 - 5,600)
Transient (n = 13)	35,000 ± 23,000	5,100 ± 2,700	21,000 ± 11,000	40,000 ± 23,000	4,400 ± 1,800	12,000 ± 4,400
(ng/g, lipid wt.)	(12,000 - 92,000)	(1,600 - 11,000)	(6,200 - 46,000)	(15,000 - 100,000)	(1,500 - 8,300)	(3,200 - 19,000)

Asterisk indicates significant concentration differences between resident and transient whales based on both wet weight and lipid weight values; Tukey-Kramer HSD test, $P < 0.05$. Letter after X ± S.D. value referes to the number of samples (other than reported value in whale form column) where OCs were detected: a (n = 10); b (n = 45); c (n = 57); d (n = 61); e (n = 62); and f (n = 63).

[a]Mean values of CBs 77, 126 and 169 are not reported because they were not detected in any killer whale biopsy blubber samples

[b]Other CB congeners (e.g., 99,149, 183, 196) may also be present (see Materials and Methods section)

[c]NR = not reported because analyte detected in fewer than 50% of total samples

[d]I = concentration of analyte not determined due to interference with coeluting compound on PYE column

The mean total CB TEQs (? TEQs) concentrations (based on wet and lipid weights) in transient animals were significantly higher than the levels in resident whales (Table 30). However, the relative proportions of dioxin-like congeners contributing to the total mean CB TEQs in resident and transient killer whales were similar (Figure 22). Because concentrations of the non-ortho-substituted CBs were below the LOD in all killer whale blubber samples, the mono-ortho substituted dioxin-like congeners were the only contributors to CB TEQs in these samples. Although the mono-ortho-substituted CB congeners contributed approximately 7% to the mean total CB concentration in resident animals and 13% to the mean ?CBs in transients, these CBs contributed 100% of the toxic potency to the mean ? TEQ in these resident and transient whales. Furthermore, CB118 was the largest contributor to the total CB TEQs in both eco-types of killer whales, contributing approximately 67% to the CB TEQs in resident animals and 72% to the CB TEQs in transients.

Table 30. Mean concentrations (X ± SD ng/g, wet weight or ng/g, lipid weight) of DDTs, HCB, ?CBs, ? DDTs, ? TEQs in biopsy blubber samples of resident killer whales from the Kenai Fjords/PrinceWilliam Sound, AK region.

	DDTs							
Whale Form	***O,p'-DDD****	***p,p'-DDD****	***p,p'-DDE****	***o,p'-DDT****	***p,p'-DDT****	***?CBs****	***?TEQs*¶***	***?DDTs****
Resident (n =	66 ± 48h	200 ± 200j	3,100 ± 4,100	380 ± 380j	97 ± 120i	3,900 ± 4,500	29 ± 33	3,800 ± 4,700

64)								
(ng/g, wet wt.)	(ND - 180)	(ND - 980)	(150 - 22,000)	(ND - 2,000)	(ND - 570)	(270 - 27,000)	(1.5 ± 150)	(190 - 26,000)
Resident (n = 64)	230 ± 170h	700 ± 570j	11,000 ± 12,000	1,400 ± 1,300j	320 ± 310i	14,000 ± 13,000	100 ± 98	13,000 ± 14,000
(ng/g, lipid wt.)	(ND - 900)	(ND - 2,300)	(670 - 56,000)	(ND - 6,500)	(ND - 1,800)	(1,100 - 65,000)	(5.9 - 470)	(730 - 64,000)
Transient (n = 13)	940 ± 880	2,700 ± 2,300	71,000 ± 54,000	6,600 ± 5,100	1,400 ± 1,000g	59,000 ± 43,000	220 ± 190	83,000 ± 63,000
(ng/g, wet wt.)	(80 - 2,800)	(110 - 9,000)	(4,300 - 210,000)	(690 - 16,000)	(ND - 3,100)	(4,900 - 140,000)	(14 - 580)	(5,200 - 240,000)
Transient (n = 13)	3,800 ± 3,300	11,000 ± 7,800	280,000 ± 180,000	26,000 ± 18,000	5,500 ± 3,200g	230,000 ± 130,000	860 ± 640	320,000 ± 210,000
(ng/g, lipid wt.)	(950 - 11,000)	(1,500 - 32,000)	(58,000 - 750,000)	(9,200 - 62,000)	(ND - 10,000)	(66,000 - 500,000)	(190 - 2,400)	(70,000 - 860,000)

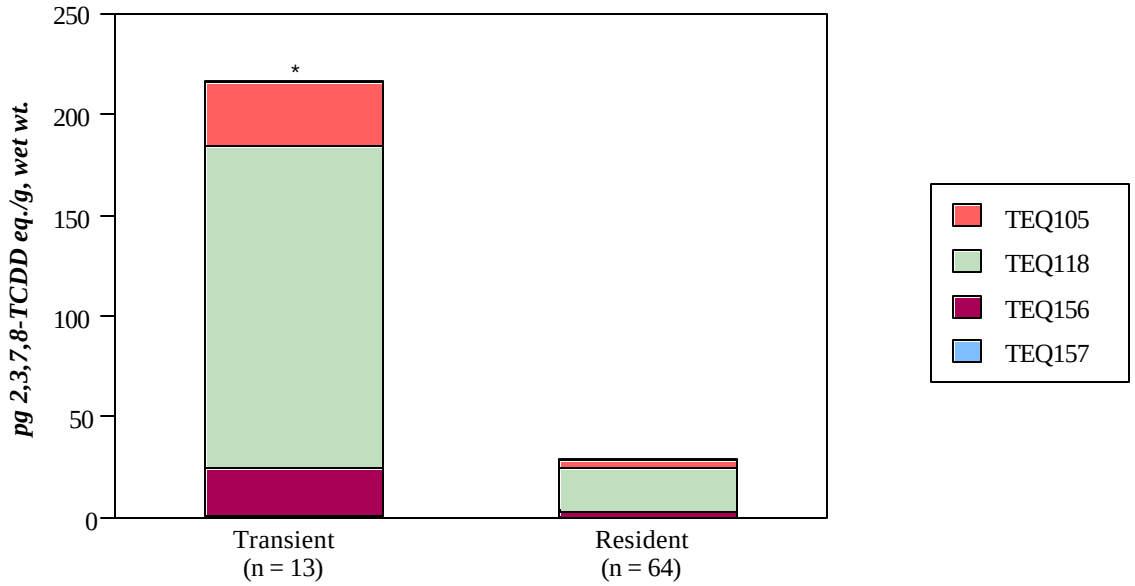
Asterisk indicates significant concentration differences between resident and transient whales based on both wet weight and lipid weight values; Tukey-Kramer HSD test, $p < 0.05$.

Letter after X ± SD value refers to the number of samples (other than reported value in whale form column) where OCs were detected: g (n=12), h (n=43),
i (n=54), j (n=60).

[a]?TEQs reported as pg/g.

[b]Lipid concentration reported as % lipid.

Figure 22: Mean CB toxic equivalents (pg TCDD eq./g, wet weight) measured in biopsy blubber samples of transient and resident killer whales from Kenai Fjords/Prince William Sound, AK. Bars with asterisks indicate significantly higher concentrations using Tukey-Kramer honestly significant difference test, $p < 0.05$.



A wide range of lipid concentrations was measured in the killer whale biopsy samples (Table 30), with levels ranging from 7.4 to 59%. The lipids measured in biopsy blubber samples consisted primarily of neutral lipids (e.g., triglycerides, non-esterified free fatty acids). The lipid concentrations

(27 ± 9.9%) of resident killer whales were not significantly different than the lipid levels in the transients (23 ± 11%).

The OC concentrations in resident killer whales were examined based on age and sex (Figure 23). For example, concentrations (based on lipid weight) of eight CB congeners (CBs 101, 105, 118, 128, 138, 153, 156 and 180) in reproductive female resident killer whales (= 13 years of age) were significantly lower than those measured in immature resident whales (male < age 15 and females < 13) or sexually mature male resident animals (= age 15). However, no differences in concentrations of these congeners were found between the immature whales and mature male animals. Similar results were observed for DDTs except p,p'-DDT. The mean concentration of p,p'-DDT in mature adult male whales was significantly higher than the mean level in reproductive females. However, we found no significant differences in mean p,p'-DDT concentrations between immature residents and mature males or immature residents and reproductive females.

The concentrations of ?CBs, ?DDTs and ?CB TEQs measured in mother - offspring groups are shown in Table 31. We found that, in both resident and transient whales, concentrations of OCs were higher in the killer whale offspring compared to the levels in the corresponding mother (Table 31). In addition, in resident whales, OC concentrations in offspring appeared to be affected by birth order. For example, a first known offspring (AE16) of AE02 contained OC levels that were approximately 9 to 20 times those in his mother and 3 to 8 times those measured in the subsequent sibling (AE20). Furthermore, we compared OC levels in sexually mature (= age 15) male resident whales (first-recruited and non-first-recruited). Overall, mean concentrations of selected CBs and DDTs in first-recruited animals were roughly an order of magnitude higher than in later-recruited whales (Figure 24). Mean concentrations of ?CBs, ?DDTs and ?CB TEQs in first-recruited whales were approximately 4.0 times those in non-first-recruited animals when based on lipid weight and 2.5 times the mean levels in the non-first-recruited whales when based on wet weight (data not shown). Stepwise regression showed that OC concentrations (based on wet and lipid weights) in sexually mature resident killer whales were more highly correlated to birth order than to age. For example, ?CBs (ng/g, lipid weight) were much more highly correlated to birth order ($\underline{p} = 0.0025$) in sexually mature resident males than to age ($\underline{p} = 0.917$). Similar results were obtained with individual CB congeners and DDT and DDT metabolites as well as ? DDTs and ?CB TEQs.

Figure 23: Mean concentrations of individual CB congeners and DDTs (ng/g, lipid weight) measured in biopsy blubber samples of resident killer whales from Kenai Fjords/Prince William Sound, AK region grouped by reproductive status [i.e., reproductive female, immature animals (both males and females), sexually mature males]. Bars with unlike letters differ significantly using Tukey-Kramer honestly significant difference test, $p < 0.05$. §CB101 coelutes with CBs 99/149/196 and possibly with other CB congeners.

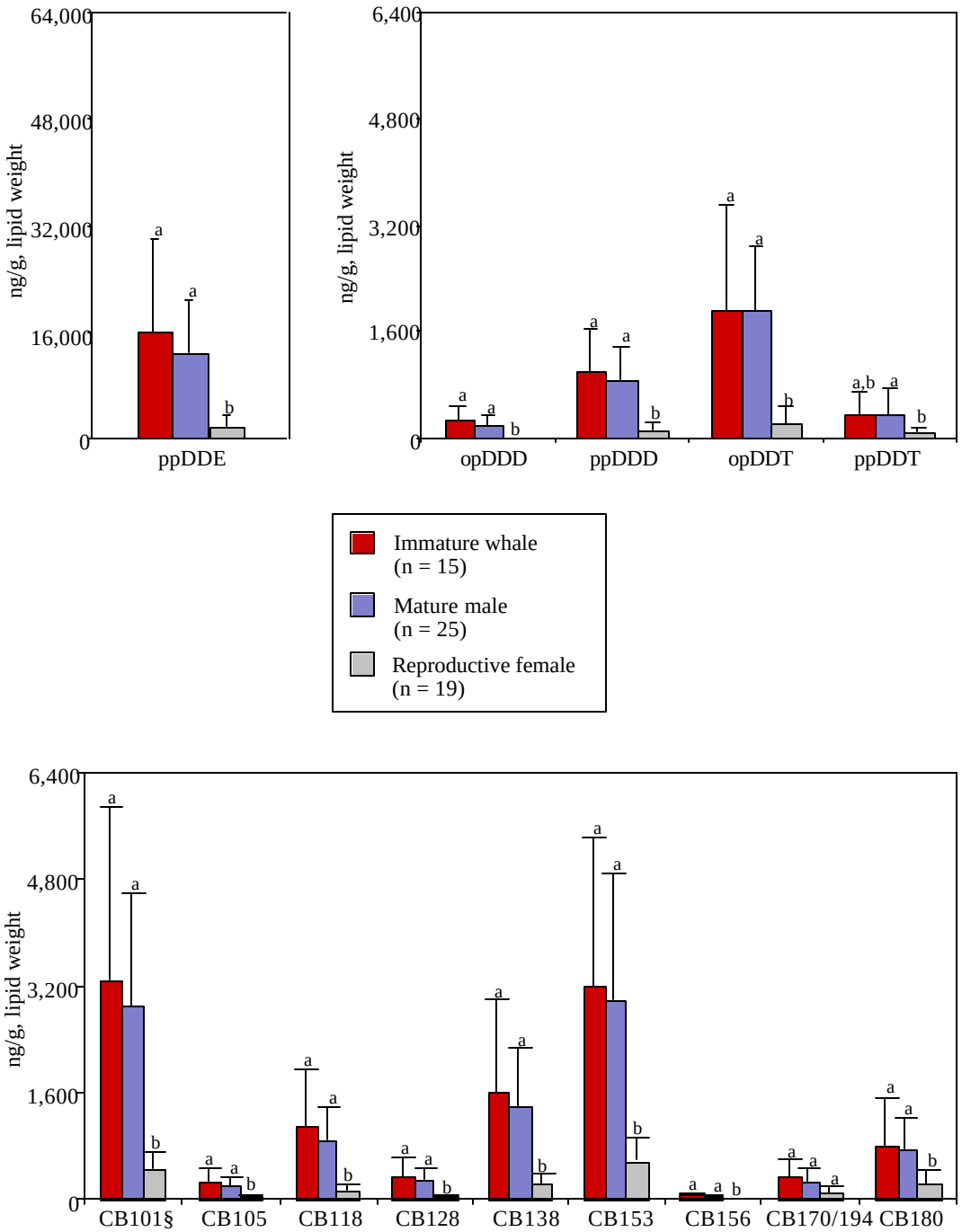


Table 31. Concentrations of total CBs, summed DDts and total CB TEQs measured in mother and offspring of Alakan killer whales from Kenai Fjords/Prince William Sound, AK.

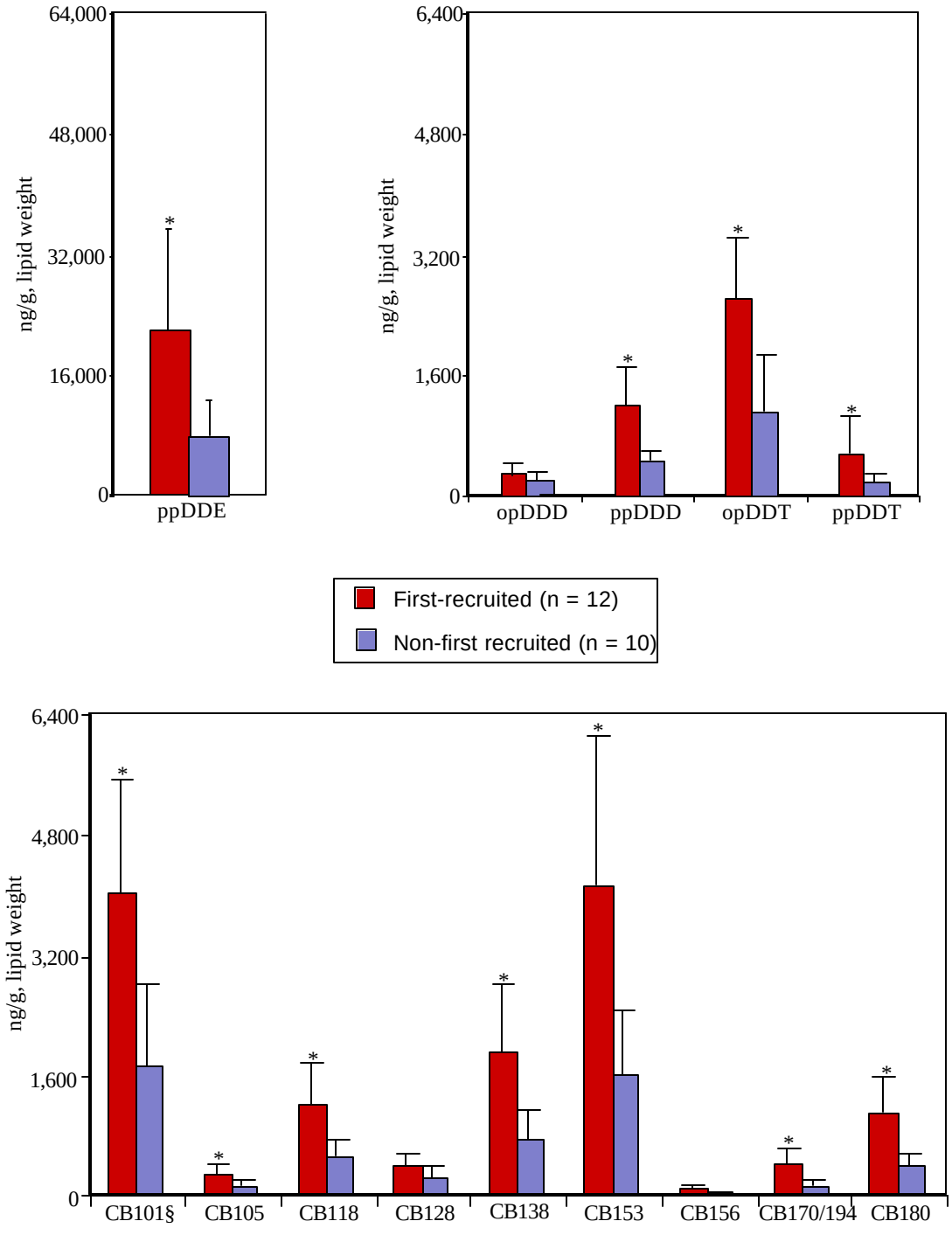
Resident pod	*Sex*[a]	*Recruitment order*[b]	*Age*[c]	*Resident pod*	*Sex*[a]	*Recruitment order*[b]	*Age*[c]
AB[d]				***AJ***[d]			
AB03	Male, sm	Unknown	28?	AJ02	Male, sm	First recruited	29?
AB04	Male, sm	Non-first recruited	28?	AJ03	Female, r	Unknown	22?
AB05	Male, sm	First recruited	> 31	AJ04	Female, r	Unknown	19?
AB10	Female, r	Unknown	> 50	AJ08	Female, r	Unknown	> 44
AB11	Male, sm	Non-first recruited	19?	AJ10	Male, sm	Non-first recruited	16?
AB17	Female, r	Unknown	> 34	AJ13	Female, r	Unknown	21?
AB24	Male, sm	First recruited	28?	AJ16	Male, sm	First recruited	28?
AB26	Female, r	Unknown	18?	AJ19	Male, sm	Non-first recruited	19?
AB27	Female, si	Unknown	15?	AJ21	Male, sm	Non-first recruited	22?
AB35	Male, sm	Non-first recruited	18?	AJ39	Female, si	Non-first recruited	2
AB39	Female, si	First recruited	11	AJ41	Female, si	Non-first recruited	1
AB40	Male, si	Non-first recruited	6				
AD[d]				***AK***[d]			
AD02	Male, sm	Unknown	30?	AK02	Female, r	Unknown	> 36
AD05	Female, r	Unknown	> 38	AK03	Female, nr	First recruited	> 53
AD13	Male, sm	First recruited	> 35	AK09	Female, si	Non-first recruited	11
AD14	Female, nr	Unknown	> 48	AK10	Female, si	Non-first recruited	7
AD16	Female, r	Unknown	> 31	AK13	Female, si	Non-first recruited	3
AD19	Male, sm	First recruited	16?	AK14	Female, si	Non-first recruited	1
AD28	Juvenile, si	Non-first recruited	3	AK15	Female, si	First recruited	1
AE[d]				***AN10***[d]			
AE01	Male, sm	First recruited	> 31	AN01	Male, sm	First recruited	> 31
AE02	Female, r	Unknown	> 20	AN03	Male, sm	First recruited	> 34
AE03	Male, sm	Non-first recruited	17?	AN07	Male, sm	Non-first recruited	26?
AE06	Male, sm	First recruited	16?	AN08	Female, r	Unknown	22?
AE09	Male, sm	First recruited	> 31	AN10	Female, r	Unknown	> 25
AE10	Female, r	Unknown	> 21	AN12	Female, r	Non-first recruited	16?
AE11	Female, r	Unknown	> 24	AN35	Female, r	Unknown	> 24
AE14	Male, sm	Unknown	18?	AN46	Male, si	Non-first recruited	5
AE15	Male, si	First recruited	6				
AE16	Male, si	First recruited	5				
AE20	Female, si	Non-first recruited	1	***AS***[d]			
				AS?	Unknown	Unknown	Unknown
AI[d]				AS12	Male	Unknown	Unknown
AI02	Male, sm	Non-first recruited	26?				

AI03	Female, r	Unknown	> 46				
AI04	Female, r	Unknown	13	***AX[d]***			
AI06	Male, sm	Non-first recruited	19?	AX31	Unknown	Unknown	Unknown

Transient group	***Sex[a]***	***Recruitment order[b]***	***Age[c]***	***Transient group***	***Sex[a]***	***Recruitment order[b]***	***Age[c]***
AT1[d]				***GOA[e]***			
AT03	Female, si	Unknown	15?	GOA	Unknown	Unknown	Unknown
AT06	Male, sm	Unknown	> 23	AT32	Male, sm	Unknown	Unknown
AT09	Female, r	Unknown	> 30	AT101	Female	Unknown	Unknown
AT10	Male, sm	Unknown	15	AT102	Female, r	Unknown	Unknown
AT13	Male, sm	Unknown	> 38	AT103	Male, si	First recruited	1
AT17	Male, sm	Unknown	> 33	AT105	Female	Unknown	Unknown
AT18	Female, nr	Unknown	> 20				

[a]*Abbreviations:* nr, non-reproductive; r, reproductive; si, sexually immature; sm, sexually mature.
[b]Putative recruitment (birth) order.
[c]Age at time of sampling
[d]Prince William Sound, AK
[e]Gulf of Alaska

Figure 24: Mean concentrations of individual CB congeners and DDTs (ng/g, lipid weight) measured in biopsy blubber samples of sexually mature male resident killer whales from Kenai Fjords/Prince William Sound, AK region grouped by birth order (first recruited and non-first recruited animals). Bars with asterisks indicate significantly higher concentrations using Tukey-Kramer HSD (honestly significant difference) test, $p < 0.05$. §CB101 coelutes with CBs 99/149/196 and possibly with other CB congeners.



Discussion

We analyzed 77 biopsy blubber samples of free-ranging killer whales from the Kenai Fjords/Prince William Sound, AK region for selected toxic organochlorines, including dioxin-like CBs. These killer whales have been extensively studied since the mid-1980's and substantial life history data (i.e., pod membership, eco-type, approximate ages, reproductive status, putative birth order) are known (Matkin et al., 1999a,b). However, few chemical contaminant data are reported for killer whales from the North Pacific (Calambokidis et al., 1984; Jarman et al., 1996; Hayteas and Duffield, 2000; Ross et al., 2000), particularly free-ranging animals from Alaska. Therefore, this unique set of biopsy blubber samples allowed us to determine the concentrations of persistent and toxic OCs in free-ranging killer whales from Kenai Fjords/Prince William Sound, AK and examine the influence of various life history parameters on OC concentrations in these animals.

The concentrations of CBs and DDTs that we measured in blubber of the Alaskan killer whales are much higher than the concentrations in blubber of various other cetaceans and pinnipeds that reside and feed in Alaskan waters (Miles et al., 1992; Varanasi et al., 1992; Varanasi et al., 1994; Lee et al., 1996; Krahn et al., 1997; O'Hara et al., 1999). For example, Krahn et al. (1999) determined the ?CBs and ?DDTs concentrations (based on wet weight) ranged from 3,770– 6,880 ng/g and 2,090 – 4,850 ng/g, respectively in adult beluga whales from three Alaskan stocks. These concentrations are comparable to those measured in resident female killer whales but were approximately an order of magnitude lower than the contaminant concentrations determined in transient whales. The OC concentrations found in the Alaskan killer whales are similar to those recently reported in pinnipeds and cetaceans that occur in more contaminated waters of the Eastern North Pacific (Lieberg-Clark et al., 1995; Jarman et al., 1996; Hong et al., 1998). For example, the ?CB levels (based on lipid weight) measured in the Alaskan transient killer whales are similar to those recently reported in biopsy blubber samples of transient killer whales from coastal waters of British Columbia (Ross et al., 2000), whereas the ?CBs concentrations (based on wet weight) determined in the Alaskan resident whales are similar to those found in blubber of harbor seal pups from Puget Sound, WA (Hong et al., 1996). However, ?CBs is, in most cases, an estimated value (unless all 209 congeners are quantitated with appropriate standards) and caution should be used when comparing ?CBs among different studies because different methods are used to calculate these values. The ?CBs reported in killer whales from British Columbia waters by Ross et al. (2000) were calculated by summing the concentrations of 136 CB congeners in killer whale biopsy samples. In contrast, the ?CBs reported in the current study were calculated by summing concentrations of selected CBs (based on individual response factor) and concentrations of other CB congeners (calculated by summing areas of peaks identified as CBs and using an average CB response factor). However, because PDA (UV) response factors for CBs vary by only ±15% or so from the average, a reasonable estimate of ?CBs is obtained using this method.

The DDT metabolite, p,p'-DDE was the OC found in the highest concentration in the Alaskan killer whale biopsy samples. Similar to our findings, p,p'-DDE was the most abundant OC measured in blubber samples of beluga whales and northern fur seals that reside in waters of the Eastern North Pacific (Mossner and Ballschmiter, 1997). Moderately chlorinated ortho-substituted CBs (i.e., CBs 153, 138) were the predominant CB congeners measured in the Alaskan killer whales. These findings are similar to those previously reported in various species of marine mammals from the Eastern North Pacific (Varanasi et al., 1994; Hong et al., 1996; Jarman et al., 1996; Mossner and Ballschmiter, 1997; Beckmen et al., 1999) as well as for pinnipeds and other cetaceans from various parts of the world (Corsolini et al., 1995; Lake et al., 1995; Gauthier et al., 1997; Weisbrod et al., 2000a). CB

congeners that contain 5 – 7 chlorine atoms make up high proportions of certain technical mixtures of CBs (e.g., Aroclor 1254) (Schulz et al., 1989; Schwartz et al., 1993). Because many of these moderately chlorinated congeners are not easily degraded in the environment or eliminated by aquatic organisms as are lower chlorinated CBs, relatively high concentrations of these congeners bioaccumulate in marine animals, especially species at the top of the marine food chain. Furthermore, certain CBs are not as readily metabolized by aquatic organisms as other chlorinated congeners relative to chlorine substitution pattern. Boon et al. (1992) report that harbor seals, cetaceans and polar bears appear to metabolize congeners with vicinal H atoms in the ortho, meta positions, even in the presence of one ortho-chlorine atom but this metabolic capability is not as apparent in ringed seals. However, irrespective of ortho-chlorine substitution, the cetacean species do not seem to metabolize CBs with vicinal H atoms in the meta, para positions as readily as the seals and polar bears. Based on these data, relatively high concentrations of certain moderately chlorinated CBs are expected to bioaccumulate in marine mammals, especially top level predators such as killer whales.
The relatively high concentrations of CBs and DDTs in Alaskan killer whales are somewhat surprising, but consistent with current information about transport of these compounds to Arctic ecosystems. Studies indicate that certain OCs primarily enter the Alaskan marine ecosystem via atmospheric transport from the lower and middle latitudes (Barrie et al., 1992; Iwata et al., 1993). These compounds can also enter the marine environment from direct input (e.g., transformer spill) into the far northern marine environment but these sources appear to be less significant than atmospheric deposition (Iwata et al., 1993; AMAP, 1998). For example, DDT is a persistent, lipophilic compound that was once widely used in the United States on agricultural crops and to control disease-carrying insects (e.g., malaria-carrying mosquitoes) and has been shown to have various toxic effects on experimental animals and wildlife (e.g., reproductive impairment, potential carcinogen). Consequently, it was banned in the U.S. in the mid-1970's and has been prohibited or restricted for use in several other countries (e.g., Canada, Sweden). However, the compound is still used to control disease-carrying insects in other regions (i.e., Southeast Asia) and appears to be deposited to the pristine Arctic and subarctic ecosystems of the eastern North Pacific via atmospheric transport (Barrie et al., 1992; Iwata et al., 1993; AMAP, 1998; Schmidt, 1998).

The CB TEQs calculated for Alaskan transient killer whales are comparable to those determined in transient killer whales from coastal waters of British Columbia (Jarman et al., 1996; Ross et al., 2000) and harbor seals from Puget Sound, WA (Hong et al., 1998). In contrast, the CB TEQ concentrations for these killer whales were much lower than the levels in striped dolphins affected by an epizootic in the Mediterranean Sea (Kannan et al., 1993), common porpoise from the Baltic Sea (Falandysz et al., 1994) and two species of dolphin from the Italian coast (Corsolini et al., 1995). The TEF values (Van den Berg et al., 1998) we used to calculate the CB TEQs are different than those used in the European dolphin studies (Kannan et al., 1993; Falandysz et al., 1994; Corsolini et al., 1995). In the Safe technique (1990), the TEF values are higher or comparable to those recommended by Van den Berg et al. (1998) and a larger number of CB congeners are used in TEQ calculations. Furthermore, the mono-ortho substituted dioxin-like congeners were the only contributors to the TEQs determined in our killer whale study because the non-ortho-substituted CB congeners (CBs 77, 126, 169) were below the LOD and PCDDs and PCDFs are not quantitated by the HPLC/PDA method. Therefore, the CB TEQ values determined in this study are conservative values. Ross et al. (2000) report the sum TEQs (based on concentrations of dioxin-like CBs, PCDDs and PCDFs) in biopsy blubber samples of northern resident killer whales from coastal British Columbia. The dioxin-like CBs contribute more than 85% to the sum TEQs in these samples, with the mono-ortho-substituted CB congeners contributing more than 80% to the sum CB TEQ (92.0 ± 1.7% in immature animals, 94.3 ±

0.8% in males and 80.35 ± 4.11 % in females) and more than 75% to the sum TEQ. Based on these data, we may be underestimating the ?CB TEQs in the Alaskan killer whale biopsy samples by approximately 6 – 20% and the sum TEQs (including CBs, dioxins, furans) by approximately 8 – 25%.

Lipid concentrations in the killer whale biopsy samples ranged widely and consisted primarily of neutral lipids (e.g., triglycerides, free fatty acids). Previous studies show that blubber of healthy cetaceans is comprised primarily of neutral lipids, such as triglycerides and nonesterified free fatty acids (Kawai et al., 1988; Tilbury et al., 1997). Lipid levels in our samples were comparable to lipid concentrations in biopsy samples of North Atlantic right whales (4.8 - 25.5%) (Woodley et al., 1991) and Northwest Atlantic right whales (mean 13 ± 18% lipid) (Weisbrod et al., 2000b), but are lower than those previously determined in non-biopsy samples of other large cetaceans (Borrell, 1993; Gauthier et al., 1997; Prudente et al., 1997; O'Hara et al., 1999). The average lipid concentration measured in necropsy blubber samples of killer whales (n = 6) collected off the coasts of British Columbia and Washington State was 91% (n = 6) (Jarman et al., 1996). This discrepancy is probably due to two factors. First, biopsy samples probably contain a higher portion of connective tissue attached to the skin and blubber than the necropsy samples, especially if collected from areas of lower lipid concentration (i.e., the base of the dorsal fin) (Woodley et al., 1991; Gauthier et al., 1997; Weisbrod et al., 2000b). Second, different quantitation methods were used in these lipid determinations. The biopsy blubber samples in our study were quantitated by TLC-FID (see Materials and Methods section) while the lipid concentrations in the Jarman et al. study (1996) were determined gravimetrically. Delbeke et al. (1995) found that lipid concentrations determined by TLC-FID were, on average, approximately half those determined by the gravimetric method, and that gravimetric lipid values were overestimated due to interference of non-lipid co-extracts. Therefore, caution should be used when comparing the lipid data from our study with lipid concentration data determined by other quantitation methods.

In this study, diet had important effects on OC accumulation in Alaskan killer whales. Transient whales contained much higher levels of OCs than did the residents. Similarly in coastal British Columbia waters, Ross et al. (2000) reported higher levels of CBs (based on lipid weight) in biopsy blubber samples of transient whales compared to those found in resident whales. Studies of feeding habits of Prince William Sound, AK resident killer whales show that these animals consume predominantly salmon and, to a lesser extent, other fish species (e.g., halibut, herring) (Matkin et al., 1999b; Saulitis et al., 2000). The principal marine mammal species consumed by transient killer whales from Prince William Sound are Dall's porpoise and harbor seals (Saulitis et al., 2000). In general, the prey species of transient whales contain higher OC levels than do resident prey. For example, ?CBs (based on wet weight) in blubber of harbor seals from Prince William Sound, AK range from 45 – 356 ng/g (Krahn et al., 1997), whereas ?CBs range from 17 - 50 ng/g in muscle of three salmon species (chum, coho, pink) collected from the same area (D. Brown, 2000 pers. comm.). Similarly, Ross et al. (2000) reported higher concentrations of CBs in prey of British Columbia transient whales (e.g., harbor seals) compared to the levels in resident whale prey (e.g., salmon) from this area. Therefore, based on their diet, transient whales would be expected to have higher concentrations of persistent contaminants than those found in residents.

Life history parameters such as age, sex and reproductive status influenced the concentrations of OCs in the killer whales. Reproductively active female killer whales contained much lower OC concentrations than sexually mature resident males or immature animals. Furthermore, killer whale offspring had higher OC concentrations than those determined in the corresponding mothers. These results are consistent with those from other marine mammal contaminant studies that report much lower OC burdens in reproductive females than in males in the same age group (Aguilar and Borrell, 1988;

Kuehl and Haebler, 1995; Krahn et al., 1999; Tilbury et al., 1999). These studies have shown that the OC concentrations in juvenile animals of both sexes increase until sexual maturity. Males continue to accumulate these lipophilic contaminants throughout their lives. In contrast, a reproductive female's OC levels decrease due to maternal transfer of lipophilic OCs to her offspring during gestation and lactation (Wagemann and Muir, 1984; Aguilar and Borrell, 1994; Beckmen et al., 1999; Krahn et al., 1999). Furthermore, in some odontocetes (e.g., killer whales, pilot whales, short-finned pilot whales), after a female reaches senescence, her OC levels again increase with age (Tanabe et al., 1987; Tilbury et al., 1999; Ross et al., 2000).

Recruitment order also appears to affect the OC levels in killer whales. For example, first-recruited (first-born) adult male resident whales contained significantly higher levels of OCs than were found in non-first-recruited males in the same age range. Lee et al. (1996) estimated that a female Steller sea lion transfers approximately 80% of her OC burden to her first-recruited offspring during lactation. In another study, it was calculated that a first-recruited offspring of a female fin whale received approximately 1g ?CBs and 1.5 g ?DDTs, but that the levels of these lipophilic contaminants transferred to subsequent offspring gradually decreased to a minimum of 0.2 g ?CBs and 0.3 g ?DDTs in old females (Aguilar and Borrell, 1994). It appears that the OC burden transferred from mother to offspring decreases as reproductive females mature, because older females that have gone through several lactation cycles have lower OC burdens (Ridgway and Reddy, 1995). These data suggest that first-recruited marine mammals are likely to be exposed to higher OC burdens than subsequent offspring and, because of these higher OC burdens, may be at higher risk of toxicological effects of these contaminants than later offspring.

We compared OC levels in killer whales to contaminant levels associated with biological and physiological effects in various mammalian species. Concentrations of ?CBs (based on lipid weight) above 77,000 ng/g, lipid are linked to reproductive dysfunction in ringed seals, harbor seals and otters and immune suppression in Rhesus monkeys (AMAP, 1998). More than 90% of the Alaskan transient killer whales contained ?CBs above this benchmark concentration whereas none of the resident animals contained ?CBs greater than 77,000 ng/g, lipid. Using experimental literature data on mink, a critical body residue (EC50) of 160 pg/g (TCDD equivalence/wet weight) is proposed for mink litter size (Leonards et al., 1995). Although none of the resident whales in this study contained ?CB TEQs above this threshold level, more than half the transients had ?CB TEQs at or above 160 pg/g, wet weight. These preliminary data suggest that the levels of OCs measured in the Alaskan killer whales could potentially cause various deleterious biological and physiological effects, such as reproductive impairment (Subramanian et al., 1987; Addison, 1989) and immune suppression (Ross et al., 1995; Ross et al., 1996). However, caution should be used in evaluation of the level of risk posed by toxic anthropogenic chemicals from this limited data set. These analyses focused only on OCs and exposure to toxic substances or other human factors, such as petroleum-related hydrocarbons, biotoxins and fishing interactions, may be affecting the health of these killer whales.

Conclusions

This study provides baseline chemical contaminant data for free-ranging killer whales in Alaskan waters for which there is little previous information. These Alaskan killer whales contain some of the highest levels of OCs reported in tissues of marine mammals from the eastern North Pacific and are comparable to killer whales from coastal waters of British Columbia. In particular, transient whales had much higher contaminant concentrations than did resident whales because they feed at a higher trophic level than do residents. In addition to diet, biological factors such as age, sex, reproductive status and

birth order also affected the concentrations of OCs determined in these animals, with elevated OC concentrations determined in sexually mature male killer whales. Furthermore, presumably first-recruited resident males had significantly higher OC levels than did those in non-first-recruited animals from the same age range. The causal factors for low reproduction and population decline of certain pods (AB pod) and groups (AT1 group) of killer whales from Prince William is not known. The low reproduction and population decline may be a natural cycle, related to human factors (e.g., fishing interactions) or to exposure to natural toxins (e.g., biotoxins) or a combination of environmental and anthropogenic factors. Exposure to toxic OCs may also be the factor or a contributing factor. The highly elevated levels warrants further examination of the relationship of OC exposure to fitness of individual killer whales and possible relationship to population declines.

OVERALL CONCLUSIONS

1. In fall 1988 AB pod numbered 36 whales; 13 whales were lost from the pod in the year and a half following the oil spill in 1989. In 2001 the pod had recovered to 26 whales, (they were not completely documented in 2002). There has been a net increase of four whales since a low of 22 members was recorded in 1995. The two calves and single mortality recorded in 2001 were in the AB25 subpod which has traveled with AJ pod since the spill. It appears that although a slow recovery is underway, it will be complete no sooner than 2015. All major resident pods were thoroughly photographed in 2001 or 2002, including the southeastern Alaskan pods AG, AF05, AF22. All are at numbers equal to or greater than prior to the spill. A population model was developed based on the years 1984-2001 for the southern Alaskan resident population that extends from southeast Alaska through PWS/Kenai Fjords and apparently on to Kodiak.

2. The AT1 population lost another individual in 2001, the young male AT10, and has the group has produced no new calves since 1984. There are now only nine individuals in this genetically unique population that numbered 22 whales prior to the spill in 1988. There is no indication of potential recovery. Although numerous factors including high contaminant levels and a depleted harbor seal population may be contributing to their lack of recovery, the nine mortalities following the *Exxon Valdez* oil spill have been the primary factor in the recent decline.

3. Genetic analysis of using both mtDNA and nuclear microsatellites has revealed two ecotypes, residents and transients, in the Kenai Fjords/Prince William Sound region. Two non-associating transient populations, the AT1 group and the Gulf of Alaska were identified as well as two interbreeding two clans of resident killer whales(AB clan and AD clan. There is no evidence of breeding within resident pods or between transient populations.

4. Acoustic analysis backs up the genetic based separations and yields a more current and fine scale examination populations and clans, which are all clearly separable by acoustics. Pods within clans are also acoustically identifiable which makes it possible to determine pod, clan, and population affiliations from recordings made from the remote hydrophone during the

winter months. A call catalogue has been developed that categorizes the calls of the pods and populations.

5. The remote hydrophone at Thumb Point documented the presence of AB, AJ, AN10, AF, AD5 and AK pods and members of the AT1 group have been recorded in Resurrection Bay in fall/winter 2000-2001 and 2001-2002. For resident killer whales patterns in winter use and in pod association in Kenai Fjords are emerging, with less mixing between clans than we see in summer months. Improvements in transmission technology have increased signal quality and reliability of the remote hydrophone, however, interruptions in the signal still occur, generally due to power supply problems and failed wind generators.

6. Resident killer whale appear to be primarily salmon feeders; prey observed are dominated by Chinook salmon in the late winter and spring and coho salmon in summer and fall; while there is no evidence of predation on pink salmon. Both the AT1 and Gulf of Alaska transients prey only on marine mammals; AT1 diet is dominated by harbor seals and Dalls porpoise, while the limited feeding data on the Gulf of Alaska transients indicates they also prey on Steller sea lions. There is evidence of only very limited predation on sea otters. AT1 transients have increased predation on porpoise as a consequence of the drastic decline in harbor seals.

7. GIS analysis indicated a partitioning of habitat in Prince William Sound between residents, which occurred in the wider entry waterways of the western Sound (Montague Strait and Knight Island Passage), and transients, which were more often found in the narrow bays and passages and were believed to reflect dietary preferences. Encounters with AE pod were more frequent over the course of this study while encounters with the declining AT1 transients declined. Encounters with other pods did not change significantly over the 1984-1996 study period.

8. Contaminant analysis found very high levels of PCBs and DDTs in both AT1 and Gulf of Alaska transient whales; these levels are high enough to affect reproduction and immune response. Contaminants were passed from mother to offspring during nursing which accounted for considerable individual variation in contaminant levels; first born offspring receiving significantly higher concentrations. Most contaminants are thought to reach the study area from southeast Asia and China in weather systems.

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

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