Exxon Valdez Oil spill Restoration Project Annual Report

Comprehensive Killer Whale Investigation

Restoration Project 96012A-1 Annual Report

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

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Comprehensive Killer Whale Investigations

Restoration Project 96012A-1 Annual Report

Study History: The current project was initiated under Restoration Project 95012a and this is the second annual report. Killer whales were previously 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. An assessment of the status of killer whales from 1984 to 1992 in Prince William Sound is provided in Matkin et *al.* (1994). The feeding habit studies, geographic information system, and genetic studies were initiated in 1995 (Matkin et al. 1996) and continued in 1996 (96012a).

Abstract: Monitoring of killer whales (Orcinus orca) was continued in 1996 using photoidentification methods. There were two births and and one death in AB pod. Nine individuals have been missing from the AT1 transient group since 1990 and one since 1991 and are presumed dead. Statistical analysis and direct observation of killer whale associations were used to examine structure of resident killer whale pods, including AB pod. Historical data on behavior and predation events was placed in a specially designed Geographic Infromation System and is being used to examine changes in killer whale behavior and the predation killer whales on the non-recovering harbor seal population. Data on transient killer whale use of the southwestern Sound was extrapolated over the entire Sound for all seasons to determine total use. Resident and transient killer were differentiated by feeding habits, the former feeding exclusively on fish and the later feeding on marine mammals. Skin samples taken by biopsy dart were used for genetic analysis that also separated resident and transient killer whale populations. Blubber samples were analyzed for contaminants and transients were found to have levels over ten times higher than residents and contaminants appeared to be passed to offspring during lactation.

Key Words: biopsy, Exxon Valdez, Geographic Information System, genetics, harbor seals, killer whales, photoidentification, Orcinus orca, predation, Prince William Sound, resident, transient

Identification data for individual whales consists of frame by frame identifications of individual whales for all exposed films. These identifications are available on computer disk upon request approved by the Exxon Valdez Oil Spill Trustee Council from Craig Matkin, North Gulf Oceanic Society (NGOS), P.O. Box 15244 Homer, Alaska (907) 235-6590. All field observations, killer whale encounter data, vessel logs and tracklines are stored in a GIS system (Arc/Info) housed at the Prince William Sound Science Center (PWSSC), P.O. Box 705 Cordova, Alaska 99574, contact Dave Scheel (907) 424-5800. This data is will be open following completion of analysis in 1999 or by request approved by the Council or by PWSSC and NGOS

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Executive Summary

Killer whales were monitored in Prince William Sound, Alaska with funding from the Exxon Valdez Oil spill Trustee Council in 1989, 1990, and 1991 (damage assessment) and in 1993 (restoration monitoring). The North Gulf Oceanic Society (NGOS) independently maintained a monitoring program in all other years since 1984 (Matkin et al. 1994). This report summarizes results of the monitoring of killer whales in Prince William Sound in 1996. The goal of the monitoring has been to obtain identification photographs of all whales in all major resident pods and transient groups on an annual basis. Photoidentification techniques (after Bigg et al. 1990) were used to identify individual whales. The current photographic database includes thousands of frames of film collected from 1984-1995 used to provide individual identifications for each encounter with whales. These data were placed in a sc parate photographic database for association analysis, examination of pod structure. Based on direct observation and statistical analysis pod structure was examined and genealogical trees were constructed containing all the individual whales in all regularly sighted resident pods. A draft manuscript this work to be published is attached in Appendix. Vital rates for AB pod and all other frequently sighted resident pods were calculated based on the photographic data and provided in tabular format.

Sighting data for the AT1 transient group in 1996 was used to update sighting histories for this group. Despite substantial field effort the number of AT1 whales sighted each year has declined following 1989. Only 11 of the original 22 whales attributed to the AT1 group were photographed in 1995. The rate of encounter with members of this group has also declined. Modeling of resighting data (1984-1995) for the individual AT1 group whales supported the hypothesis that the missing whales are dead or have permanently emigrated from Prince William Sound.

Data on killer whale behavior and predation events were recorded in a standard format during all years of the monitoring program. Vessel tracks and maps of whale movements were also maintained. Data entry into the GIS database was completed in 1996 for all NGOS killer whale records from 1984 to 1996, including a total of 1508 boat-days of search effort and 663 encounters with whales . These data were error-checked for consistency with the original data sheets recorded in the field.

A 2 km by 2 km grid was overlaid on the Prince William Sound to examine the distribution of search effort; and the area was then divided into seven zones of approximately equal search intensity. The highest search intensity occurred in Knight Island Passage, with more moderate effort distributed throughout Prince William Sound. Encounter rates were calculated as encounters per 100 km of searching. Encounters with transients were more likely around southwest Knight Island near base camp and less likely around southeast Knight Island compared to the rest of the Sound. The analyses of encounters per unit effort indicate that transient use of other areas of the Sound is not significantly lower than in the southwest. There also seem to be no reason to expect that transient use is lower during other seasons than during the field season. It is therefore

appropriate to extrapolate transient killer whale use over the remainder of the Sound and across all seasons (areas and times when little searching was conducted) to arrive at the total use of the Sound by killer whales.

It was calculated that transient killer whales used the study area between 59 and 264 whale-days during field seasons ranging from 29 to 129 days in length. Most of this use was in the bays and passes of southwest Prince William Sound, including Knight Island Passage and Montague Straight.

Additional observation of killer whale predation and collection of killer whale prey items occurred in 1996. Predation information is included as part of the GIS data base and analysis of prey items are being used to determine the specific components of the killer whale diet. Results indicate a clear dietary separation between resident (fish eating) and transient (marine mammal eating) killer whales. Harbor seals and Dall's porpoise are important prey items for transient killer whales in the April-October period comprising 30% and 44% respectively of the observed kills. Coho salmon are important prey for resident killer whales from July-September. A draft manuscript for publication was prepared in 1996 (Appendix).

Biopsy tissue sampling for genetic analysis and contaminant analysis occurred in 1994 and 1995 using a biopsy dart system and field techniques developed by Barrett-Lennard et al. (1996). An additional 5 tissue samples from individually identified, free ranging killer whales were collected in 1996 during the monitoring program. A total of 53 full-sized samples have been collected from resident and transient killer whales. Mitochondrial DNA (mtDNA) from each sample was amplified using the polymerase chain reaction (PCR). Direct sequencing of the d loop of the mitochondrial genome was used to determine differences between proposed Prince William Sound killer whale populations. Genetic analysis using mtDNA techniques was completed in FY96 and revealed four haplotypes in Prince William Sound killer whales. Two were transient (marine mammal eating), the AT1 group and the Gulf of Alaska transients, and two were resident (fish eating) types. The analysis was carried out concurrently with a comprehensive genetic analysis of British Columbia killer whale stocks. The two Prince William Sound resident haplotypes were genetically similar to the northern and southern resident haplotypes in British Columbia, while both transient haplotypes identified in Prince William Sound were genetically separated from the single transient haplotype identified in British Columbia.

The subcutaneous portion of the 1995 biopsy samples were forwarded to Dr. Graeme Worthy for lipid/fatty acid analysis (separate project). However the subcutaneous portion of the 1994 (collected under private funding) and 1996 samples were analyzed for environmental contaminants at the NMFS Environmental Contaminant Laboratory, Seattle. Contaminant levels in both transient populations (AT1 group and GOA transients was over ten times higher than in the resident population and reflects the differing trophic position of prey for residents and transients. Contaminants appear to be passed from mothers to offspring; reproductive females had the lowest contaminant levels in all populations and first born offspring appear to have the highest contaminant levels.

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 from AB pod and there was no recruitment into the pod. The rate of mortality observed in this pod after the oil spill (19% in 1989 and 21% in 1990) far exceeds rates recorded over the past 11 years for the other resident pods in Prince William Sound or over the past 20 years for 19 resident pods in British Columbia and Washington State (Balcomb et *al.* 1982, Bigg 1982, Olesiuk et *al.* 1990, Matkin et *al.* 1994). Since the time of spill the social structure within AB pod has continued to show signs of deterioration. Subgroups have traveled independently of the pod, and pod members have not consistently traveled with closest relatives. AB pod was seen less frequently following the spill. Prior to spill AB pod was the most frequently encountered resident pod in Prince William Sound (Matkin et *al.* 1994). Although AB ;pod had a net gain of one whale in 1996, it still numbers only 23 whales. There were 36 whales in AB pod in 1988 prior to the spill.

Eleven of the 22 whales from the transient AT1 group have not been observed or photodocumented for at least five years despite extensive field effort. While mortalities in transient groups cannot be confirmed with the same certainty as for residents, there is an increasing likelihood that these whales are dead or have permanently emigrated from the Sound.

The AB pod and AT1 group possibly were injured due to the effects of the *Exxon Valdez* oil spill and that they do not appear to be recovering. Numbers of whales in other well-documented resident pods have increased during the same period. Annual photographic monitoring has been the most effective tool in determination of the recovery status of AB pod and the AT1 group and the status of the entire Prince William Sound killer whale population (Matkin et al. 1994). This project continues using photoidentification to monitor changes in resident killer whale pods (including AB pod) and the AT1 transient group in Prince William Sound.

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. At least 300 harbor seals were killed at the time of spill and the harbor seal population continues to decline. It appears that there are two types of killer whales in Prince William Sound, only one (transients) has been observed preying on marine mammals. Scale samples and bits of marine mammal flesh were collected when possible during feeding bouts, providing positive evidence of predation and of prey type. Tabulation of predation events indicated harbor seals and Dall's porpoise are the primary food items of transient killer whales from April to October. Resident killer whales appear to select coho salmon from mixed schools during the July to September period. A draft manuscript detailing feeding behavior has been prepared (Appendix).

This project examines harbor seal predation parameters using historical killer whale behavioral data in a GIS framework Historical data on killer whales collected by North Gulf Oceanic Society (NGOS) from 1984 to 1996 includes six years of pre-EVOS data, and provides the best available record of how killer whale habits may have changed following the oil spill. One goal of this project is to provide geographically-referenced analyses of this data to address questions of interest to restoration management. To accomplish this, a geographic information system (GIS) database was designed and the data entered into a computer from hand-written data sheets.

This data is the best record available to answer questions about the impact of the EVOS on whale diet or habitat use, to examine the impact of whales on other injured species (especially harbor seals), to maximize the utility of continued data collection through improved sampling protocol, and to corroborate results from studies of whale biology relying on alternative methods (i.e. stable isotope and fatty acid analysis).

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. and habitat data is in the process of being analyzed to detail demographics and spatial distributions.

Predation of harbor seals by killer whales is considered one probable factor that may limit the recovery of seals. This database is being used to estimate whether whale predation rates on harbor seals may have increased over time, and to estimate where and how many seals are killed by whales. These results can then be incorporated into models of harbor seal population dynamics (project 064, seal trophics).

This project also examined the separation of marine mammal eating and fish eating killer whales is examined using this behavioral data and genetic analysis. Full sized biopsy samples have been obtained from 54 whales. Genetic material was obtained using lightweight biopsy darts (Barrett-Lennard et al 1996). The genetic analysis in FY96 focused on mitochondrial DNA (mtDNA). 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.

Subcutaneous material collected was supplied for lipid/fatty acid analysis in 1995, but previous and additional samples are being used for contaminant analysis. Contaminant analysis is being 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.

Objectives

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

2. To examine resident killer whale associations by direct observation and statistical analysis, to construct genealogical trees and examine the structure of damaged and undamaged pods

3. To complete input of historical and 1996 observational data into the specially designed GIS system at the Prince William Sound Science Center

4. To estimate the sampling effort, to provide a measure of sightings per unit effort

5. To make numerical estimates of killer whale predation rates on harbor seals

6. To continue field observations of killer whale behavior and predation

7. To determine the behavioral and genetic separation of the putative populations of killer whales in Prince William Sound

8. To examine contaminant levels in Prince William Sound killer whales

Methods

Field Methodology

Most field work for the 1996 photoidentification study was conducted from the *Whale 2*, a 7.9m live-aboard vessel powered by a 165 hp diesel engine with inboard/outboard drive. This vessel centered its range in Montague Strait and lower Knight Island Passage but also made occasional searches in northwestern PWS and Upper Knight Island Passage. The 12.8 m vessel *Lucky Star* was used as a supply boat, hauling fuel, food, and equipment to the study area.

N.G.O.S. biologists on the *Whale 1* (a 7.8 m light motor-sail vessel with 50hp outboard) also photographed killer whales and kept data sheets during surveys directed at humpback whale photoidentification. The field time and killer whale encounters for these vessels were included in the GIS data base and used in our analysis.

Researchers attempted to maximize the number of contacts with each killer whale pod to insure sufficient photographs of each individual within the pod. Searches for whales were not random, but based on current and historical sighting information.

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.

Searches were centered in areas that had produced the most encounters with killer whales in the past. In all years whales were found visually, or by listening for killer whale calls with a directional hydrophone, or by responding to VHF radio calls from other vessels. Regular requests for recent killer whale sightings were made on hailing Channel 16 VHF. Photographs for individual identification were taken of the port side of each whale showing details of the dorsal fin and white saddle patch. Photographs were taken at no less than 1/1000 sec using Ilford HP5, a high speed black and white film, exposed at 1600 ASA. A Nikon 8008 autofocus camera with internal motor drive and a 300 mm f4.5 autofocus lens was used. When whales were encountered, researchers systematically moved from one subgroup (or individual) to the next keeping track of the whales photographed. If possible individual whales were photographed several times during each encounter to insure an adequate identification photograph. Whales were followed until all whales were photographed or until weather and/or darkness made photography impractical

A vessel log and chart of the vessel track were kept for each day the research vessels operated. Similar logs were kept for all previous study years will be used in the GIS format to estimate effort (Matkin et al 1996). On these logs the elapsed time and distance traveled were recorded and vessel track was plotted. Record was made of time and location of all whale sightings and weather and sea state noted at regular intervals.

Specifics of each encounter with killer whales were recorded on standardized data forms that have been used since 1984. These forms were modified in 1995 to improve collection of data for GIS input (Matkin et al 1996). Data recorded included date, time, duration, and location of the encounter. Rolls of film exposed and the estimated number of whales photographed also were recorded. A chart of the whales' trackline during the encounter was completed 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. Only one or a few sightings were recorded on any field day, but encounters with whales averaged from 3-6 hours, providing considerable behavioral information (travel rates, duration of feeding bouts, etc.). On each sheet the path of the vessel (LOG) or whales (ENCOUNTER) was recorded on a sketch map.

Directed observations of feeding behavior and identification and collection of prev of killer whales were made when possible during the 1996 fieldwork. Prey identification and collection from prior years was also available and used in our analysis. 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 collected. This included instances where evidence was not observed but a kill was suspected or when potential prev exhibited fright or flight response or other strong behavioral reaction to killer whales. Harassment was demonstrated by behaviors such as flipper slapping and lobtailing by humpback whales and fleeing behavior by small cetaceans, pinnepeds, or mustelids. When predation on fish was observed, fish scales from the site of fish kills by killer whales were collected and later identified by species. Slides were individually mounted and identifications were made by a laboratory specializing in fish scale aging and identification. 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.

Tissue samples were collected opportunistically in 1996. There was no directed biopsy program. Samples were collected using a pneumatic rifle and custom-designed biopsy darts (biopsy system as described in 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 dart (approx. 10 cm long by 1.2cm dia.) was fitted with a beveled tubular sterile stainless steel tip that took a small core of skin and blubber (approximately 1.6cm long and 0.5cm dia.). The sterilized dart is fired from a range of 16-20m. The dart hit the animal in the upper back, excised a small tissue sample and bounced off. The dart floated with sample contained until retrieved.

From the biopsy samples the epidermis, which was heavily pigmented, was separated aseptically from the other layers with a scalpel as soon as the dart was retrieved from the water. The dermal sample was used as a source of DNA, and was stored at 4 deg C. in a sterile 1.7 ml cryovial containing 1.2 ml of an autoclaved solution of 20% DMSO and 80% sodium chloride saturated double distilled water (for properties of storage solution see Amos and Hoelzel, 1991). The dermis and hypodermis were made up primarily of collagen and lipid, respectively, and were frozen in autoclaved, solventwashed vials for contaminant analysis.

Data Analysis

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

The alphanumeric code used to label each individual was based on Leatherwood et. *al.* (1984) and Heise et *al.* (1992). 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 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. The actual number of whales identified from photographs and pods of whales present for each encounter was extracted from the photographic database and included with each encounter entered in the GIS database.

New calves were already present when fieldwork began 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. Although young calves may travel with other individuals at times, a majority of time is spent with the mother as demonstrated by association analysis of identification photographs from repeated encounters (Bigg et *al.* 1990). The white saddle patch of calves generally does not develop for several years, but other scars and marks including the shape of the white eye patch are used to reliably reidentify calves.

If a whale from a resident pod is not photographed swimming alongside other members of its matrilineal group (see Appendix 2) during repeated encounters over the course the summer field season it is considered missing. If it is again missing during the repeated encounters in the following summer season it is considered dead. No individual resident whale consistently missing during repeated encounters with its pod and 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 (Bigg et *al.* 1990, Matkin et *al.* 1994). Subgroups of resident pods may travel separately from the pod 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 recorded dead 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 for several years cannot necessarily be considered dead.

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 given year

NP = number of whales present in a pod at

end of 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 for all pods for all years.

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 offspring. Whales of adult conformation at the beginning of the study that had not calved since 1983 and were not accompanied by a juvenile(s) were considered as possibly post-reproductive. Exact ages of whales could be determined only for whales born since 1983. Juveniles born before 1984 were given approximate ages by comparing the relative size of the whale and development of saddle patch and dorsal fin in photographs from 1984. Males are readily identified at about 15 years of age as their dorsal fin grows taller and less falcate than females. At sexual maturity fin height will exceed width by at 1.4 times (Olesiuk et. *al.* 1990). The fin continues to grow until physical maturity (about 21 years of age). A more thorough treatment of estimating ages of whales in provided in Appendix 2 a draft association analysis manuscript).

Subgroupings (matrilineal groups) of whales within resident pods were determined by direct observation, examination of photographic negatives, and by statistical analysis of association patterns in photographic data. This is throrougly described in Appendix 2)

Sighting data for individual transient killer whales was recorded and the summary table for the AT1 transient group updated with 1996 sighting data for each individual.

The cumulative number of different AT1 individuals was plotted against effort (days in the field) for the 1996 season and compared with similar data averaged for 1984-89 and 1990-1995.

Geographic Information System (GIS) based analysis

The GIS database on killer whales stores spatially referenced data (in this case, vessel or whale paths) associated with tabular data. Two sets of data layers are maintained, the first consisting of vessel log records documenting the search effort; the second consisting of whale encounter records documenting each sighting of killer whales. Details of the design of the database were included in Matkin et. al. (1996), however we include the data dictionary (Appendix 1) in this report again to document a few modifications of the tabular data. Data were entered and error checked by Eva Saulitis, who was present in the field for most years of the study. While all data collected to date have been entered, we limited our first analyses to the AT1 and GOA transient groups, because of our focus on understanding killer whale predation on harbor seals.

Search effort was measured as kilometers of vessel track, and represented the distance that each vessel traversed. To examine search effort, we divided the Sound into a 2-km-by-2-km grid (Figure 1) and calculated effort (kilometers searched) within each grid cell for each year. This provided a map of the intensity of search effort, with a resolution of 2 km squared (color Figure 2).

We then determined the start point for each encounter with killer whales and tabulated the number of encounters in each year that started within each grid cell. This number of encounters, divided by the kilometers of effort for that grid cell, is the encounters-per-unit-effort. As this is an indicator of the ease of finding whales in a particular location, we assume it indicates how much whales use different areas of the Sound. Calculated at this resolution, however, most cells contained either zero or one encounters. Thus, sightings-per-unit-effort was inversely related to effort, and we concluded that sighting data were too sparse to reflect the habits of AT and GOA groups at this resolution.

We therefore divided the study area into seven zones (Fig. 3), determined by visual inspection of Fig. 2 (color) to have approximately even search effort. That is, effort throughout Zone 1 is uniformly high while effort throughout Zone 7 is typically low (compare Figs. 2 and 3). Areas of sparse search effort were made into larger zones to increase the sample size within a zone. Effort and sightings were recalculated for each Zone, and analyses of area use by whales were based on zones.

We tabulated the number of whales present at each encounter. All entries were checked against photographic records to ensure that the pod-ID and numbers of whales present were correct. For the major resident pods (AB, AE, AI, AJ, AK, AN, AN10, AN20), not all of the individuals in the pod may have been in the immediate area during an encounter. However, because of the results of the association analyses (this report), we feel that any remaining individuals not in the immediate area would be nearby. Thus for purposes of determining number of whales in the area, the total size of a major resident pod is the most reasonable estimate. For encounters with lesser-known resident pods, we used the number of whales photographed as the number present, since the total size of the pod may be unknown. This may be an under-estimate in encounters where the

field estimate is considerably higher than the number of individuals photographed (i.e. some whales may have been missed by the photographers). Finally, because transient type whales do not always travel together (see association analyses), we used the number photographed as the measure of transient group size. The method of estimating group size is recorded in the database Conf-type field (see Appendix 1) for each record. For transients, these group sizes were summed across encounters to arrive at the number of whale-days that whales were in the Sound, as documented from encounter records. NGOS researchers also keep a record of radio calls from other vessels that report sightings of killer whales. The radio logs report the date, time, location, number of whales seen, and vessel making the report. Although the pod or group ID were not known, we inferred that the reported whales were transient type whales if the group was small (< 4 whales) and located close to shore in bays or passages. We were therefore able to document additional use of the Sound by transient whales from the radio logs.

Using the two separate records, it is possible to estimate the probability that transient whales were in the study area on a particular day and not detected. Whales were recorded in encounters on E days, and in the radio logs on R days, and in both on B days. Hence, whales were known to be present in the area on a total of T = (E + R - B) days; but were missed by the radio logs on some days and missed by researchers on others. Whales were detected by researchers on (E/T) days; and detected by the radio logs on (R/T) days. Thus, the probability that whales were actually present but not recorded in either data set is:

P = (1 - E/T) * (1 - R/T)

and the number of days a transient group was likely present but not recorded is:

M = P * number of search days on which whales were not detected

For several years, no radio logs were available (1984, 1986-1988, 1996) or very few radio reports were recorded (1993). For these years, we could not calculate the probability that transient whales were present but not recorded. Instead, we assumed that the chance researchers would miss transients was equal to 0.37, the average value of that probability calculated from years in which there were more than 20 radio reports during the field season (1985, 1989-1995). The number of whale-days represented in radio reports and the present-but-missed estimation was calculated as 4.57 * (M + R), where 4.57 is the average group size for transient whales in Prince William Sound (Saulitis et. al. unpublished ms). To arrive at the estimated total whale-days of use for each year, we summed encounter-whale-days with the whale-days from radio reports and present-but-missed estimates. This total is the estimated use of the study area by transient whales during the study period and thus represents use by potential predators on harbor seals.

To conduct the mtDNA sequencing, we (1) amplified the entire mtDNA D-loop region with the polymerase chain reaction (PCR), using custom-designed primers based on published mtDNA sequences of other cetacean species (eg Arnason et al 1991). The sequences were checked by eye and then aligned using the program CLUSTRAL W. We used a maximum likelihood inference method (reviewed in Swofford et al. 1996) to develop and evaluate hypotheses concerning historical relationships between killer whale groups. Details of the analysis are given in Appendix 4, a draft journal manuscript. Killer whale blubber samples were analyzed for selected chlorinated hydrocarbons (e.g., dioxin-like CBs, DDTs) using rapid high permormance liquid chromatography/photodiode array (HPLC/PDA) method. A blubber sample (0.1- 0.3g wet weight), 20ml hexane/pentane(1:1v/v) 5g sodium sulfate and the surrogate standard (1,7,8- trichlorodibenzo-p-diaoxin; 250ng) were homogenized, decentrifuged and decanted into a concentrator tube. The homogenization process was repeated, the extracts were combined and evaporated to 1 ml. the sample extract was loaded onto gravity-flow cleanup column (which contained a glass wool plug, silica gel, basic silica gel and acidic silica gel) to separate the CBs from other interfering compounds (i.e., lipids, aromatic hydrocarbons). The CBs were eluted from the cleanup column with 14ml hexane/methylene chloride (1:1 v/v) and collected into a concentrator tube. The HPLC internal standard was added to each sample (1,2,3, 4- tetrachlorodibenzo-p-dioxin; 250ng) and the solvent volume was reduced to 150 ul.

Eleven dioxin like congeners (CBs 77, 81, 105,118,126,156,157,169,170,180,189) were resolved from other selected CBs (CBs 101,128,138 and 153) and chlorijnated hydrocarbons (e.g., p,p'-DDD, p,p'DDE, p,p'-DDT) by HPLC on 2 (1-pyrenyl) ethyldimethylsilylated silica (PYE) analytical columns (connected in series) cooled to 9 degrees C and were detected with a PDA detector (Krahn *et al.*, 1994). These analytes were identified by comparing their UV spectra (200-310 nm) and retention times to those of reference standards in a library. Compound purity was confirmed by comparing UV spectra collected for a peak to the apex spectrum.

Results

The *Lucky Star* completed 15 survey days, including 2 partial survey days supplying the Whale 2. The *Whale2* completed 52 survey days with four weather days. The *Whale 1* completed 25 survey days. A total of 92 survey days (LOG entries) were entered in the GIS database for 1996 (Table 1).

Killer whales were encountered on 32 occasions in 1996 (Table 2), with 22 encounters with resident pods, 4 encounters with the AT1 transient group, 5 encounters with Gulf of Alaska transients and one encounter with possible new transients of uncertain affiliation. Researchers traveled approximately 856 km with whales during these encounters. A total of 179 identifiable killer whales were photographed in 1996. Of these, 162 were resident whales that were attributed to pods. Additionally, six AT1 group whales and 11 other transient whales were identified in 1996.

Members of the AT1 transient group photographed only in at the end of July and early August. There was no fieldwork in the April-June and September-October periods when sightings of these whales have been more frequent. Resident whales were most frequently encountered in late July and August although sighting rates were lower this year than for most other years (Figure 4). The most killer whale encounters (19), occurred in August and were primarily with resident pods. All encounters of three or more resident pods ("superpods") occurred in late July or August.

Resident pods

The total number of whales in well-documented resident pods other than AB pod has increased from 78 to 87 whales from 1992 through 1996, while AB pod has declined from 26 whales to 23 whales in that same time period (Figure 5). All resident pods have increased since 1984 except AB pod (Figure 6)

From 1995 to 1996 AB pod had a net increase of one individual, due to recruitment of two calves and one mortality. The mortality (to be confirmed in 1997) was AB4, an adult male in the AB10 subpod. This whale was an a fully mature adult male (at least 21 years of age) in 1984. The mimimum age at death for AB4 was estimated at 32 years. Two new calves AB50 (mother AB26) and AB51 (mother AB25) were produced in AB pod. AB25 and calf AB51 are members of the AB pod subgroup that has been traveling with AJ pod since 1993.

A total of four calves were recruited into the other five well-known resident pods in 1995/96 (Table 3). This included AI7, offspring of AI4. AI4 was born in 1984, the first year of the study and was 12 years of age at the time of the birth. This is the first calf recruited to a female born during the study. Two new calves (AN50,AN51) were recruited in AN10 pod and one new calf (AJ39) recruited to AJ pod. There were no mortalities observed in these other resident pods, however, AJ pod and AK pod were not completely photographed. Annual mortality and recruitment rates were calculated by pod and are listed in Table 3.

Transient whales

A total of 6 of the original 22 AT1 group whales was photographed in 1996. These were AT1, AT6, AT9, AT10, AT14, and AT18. AT12 has now been missing for five years and is suspected dead. It is now suspected that 11 whales in the AT1 group are dead. Since 1989 the number of individuals identified has been 12 or less despite a field effort that exceeded 200 vessel days in 1990 and 1991 (Figure 7). There were no new calves identified in 1996 in the AT1 group 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 1990-1995 was considerably lower than for 1984-1989. (Figure 7) In 1996 the individuals sighted per effort was below the average for both 1990-1995 and for 1984-1989. Both before and after 1989 there was an initial high rate of discovery of nonphotographed AT1 individuals in the first 60 days of each field season followed by a sharp reduction of new whale discoveries despite repeated encounters with AT whales. In 1996 there was an atypically low rate of discovery of unphotographed AT1 whales due to a lack of encounters in early July (Figure 7a).

A total of 202 whales photographed between 1984-1995 were grouped by observation and association analysis into 9 pods. The individuals were placed in 39 maternal genealogical trees. An additional 4 calves were observed in 1996 and placed in

the genealogical trees based only on field observations. (A draft journal manuscript detailing association analysis results is found in Appendix 2)

Data entry into the GIS database was completed in 1996 for all NGOS killer whale records from 1984 to 1996, including a total of 1508 boat-days of search effort and 663 encounters with whales (Table 1). All entries were checked against photographic records to ensure that the pod-ID and numbers of whales present were correct.

We also examined NOAA/NMFS killer whale data for 1993 (received from David Bain) including 19 encounters when NGOS was not present (potentially unique data). However, while we appreciate the potential value of these data, we felt that we could not easily add them to our database because NOAA/NMFS field effort was focused intently on photo-id, and did not provide the behavioral and location details that are the focus of the GIS database.

Most searching was conducted in Montague Straight, and Knight Island Passage (zones 1, 2, and 5 in Figure 3) in most years (Figuress. 8-10). Of these, Zones 1 and 2 are small and hence had the highest overall coverage. Zone 5 is larger with comparatively high effort; while Zone 3 is small with lower absolute values of search. Hence, Zones 3 and 5 were each covered at moderate and comparable levels for their area. Zones 4, 6 and 7 are all large and received sparse effort (Figures. 2-3, 8-10).

We considered the AT1 group and GOA transients separately. Although our focus this year was on transients, we include resident pods in these analyses for comparison. AT1 group was encountered more frequently in 1988-1989, and less frequently in 1991, 1994, and 1996 (Figure 8). Chi-squared test, c = 35.3, df = 12, p < 0.001). AT1 group was encountered more frequently in Zone 1 and less frequently in Zones 2 and 4 (Figures 8,12). Chi-squared test, c = 33.3, df = 6, p < 0.001). The effects of zones and years on encounter rates with AT1s were not independent: AT1s were more often encountered in Zone 1 in 1988-89 and in 1992 and were less often encountered in Zone 2 in 1992 (Chi-squared test, c = 129.4, df = 72, p < 0.001). Finally, the AT1s were encountered overall more frequently in the 1980s than in the 1990s (Figure 8. Chi-squared test, c = 14.8, df = 1, p < 0.001).

GOA transient groups were encountered more frequently in 1987 and 1995-1996, but less frequently in 1991-1992 (Figures 9,11). Chi-squared test, c = 26.1, df = 12, p < 0.05). GOA groups were encountered more frequently in Zone 5 but less frequently in Zones 1 and 7 (Figures. Chi-squared test, c = 18.9, df = 6, p < 0.01). However, the effects of zones and years on encounter rates with GOAs were independent (Likelyhoodratio chi-squared test, G2 = 35.1, df = 72, p > 0.05). Finally, differences between the 1980s and 1990s in overall encounter rates with the GOA transients were not significant (Fig. 9. Chi-squared test, c = 1.7, df = 1, p > 0.05).

For comparison, resident groups were encountered more frequently in 1985-1987, but less frequently in 1989-1990 (Figures 10,11). Chi-squared test, c = 65.4, df = 12, p < 0.001). Residents were encountered more frequently in Zones 1-2 but less frequently in Zones 3 and 6 (Figures. 10,12). Chi-squared test, c = 66.0, df = 6, p < 0.001). The

effects of zones and years on encounter rates with residents were not independent: there were more encounters in Zone 1 in 1984-86 and in Zone 2 in 1993 and 1995. There were fewer encounters Zone 5 in 1990 (Chi-squared test, c = 218.3, df = 72, p < 0.001). Finally, residents were encountered overall more frequently in the 1980s than in the 1990s (Figure 10) Chi-squared test, c = 6.34, df = 1, p < 0.05), due to high encounter rates that were limited to the mid-eighties rather than continuous in 1984-1989.

Comparing radio reports and encounters for particular days allows us to calculate the probability that transient whales that were present in the study area would be detected. The AT1s could not be distinguished from GOA transients based on the radio reports, so these calculations could not be done separately for the two sets of whales. Researchers on average detected transient whales on 63% of the days they were known to be present in the study area (Table 4. N = 7 years with radio reports). The probability that transient whales in the study area were not detected by either researchers or radio reports varied from 7-22%. The average number of transient whales per day using the study area during the field season ranged from 1.54-2.64; when multiplied by 364 days per year, this gives a range of 562-964 transient killer whale days per year in the portion of the Sound covered by researchers (Table 4). The highest use rate occurred in 1988 and the lowest rates occurred in 1985 and 1995. There were no apparent long term-trends in use.

This use estimate is based on sampling that is disproportionaly weighted to the southwest corner of the Sound (the study area); and is therefore too low to represent transient use of the whole Sound. However, the area of most intense sampling does apparently include the areas most heavily used by AT1s (Zone 1) and GOAs (Zone 5). We suggest that the study may document about one half of transient use in the Sound, even though the area of intensive sampling comprises less than 50% of the Sound. Therefore, we adjusted the annual study area transient whale days upward by a factor of two to estimate annual Sound-wide transient whale days.

Due to the difficulty of recognizing underwater hunting and feeding behaviors, marine mammal kills were not regularly recorded in the early years of the study. However, beginning in 1988, marine mammal kills were more reliably observed. Between 1988 and 1996, 34 kills of marine mammals were observed, and the species of prey was determined for 31 of these. These observations represent a sample of the killer whale's diet that can be used to estimate the proportion of the diet obtained from each prey species (Table 5). Most kills (28 of 31, or 90%) were of either Dall's porpoise or harbor seals, and the bulk of the diet comes from Dall's porpoises, as these were the larger prey.

Killer Whale Predation

A total of 19 salmon scale samples (17 could be identified by species) were collected in 1996 from the sites of fish kills by resident killer whales. Sixteen were coho samon (*Onchorhynchus kisutz*) and one was a chum salmon (*Onchorhynchus keta*). Only resident killer whales were observed preying on fish. There were 5 observations of predation on and six observations of harassment of marine mammals by transient killer whales. Dalls poropoise (*Phocoenoides dalli*) were preyed upon on 2 occasions, unidentified porpoises on two occasions and an unidentified marine mammal on one

occasion. Appendix 3, is a draft journal submission that details the methods and results and discusses our predation studies.

Genetics

When the mtDNA sequences of 40 Prince William Sound killer whales were aligned, we found eight variable nucleotide sites, comprising one insertion/deletion and seven purine-purine or pyrimidine-pyrimidine transitions. The differences at these sites separated the sequences into four haplotypes. One of the haplotypes was common to all members of the Gulf of Alaska group, the second to all members of the AT1 transient group, the third to all members of 6 resident pods, and the fourth to all members of four resident pods. Detailed results of the genetic analysis are presented in the draft journal submission attached as Appendix 4

Environmental contaminants

Biopsy tissue from the outer blubber layer of 31 individual killer whales was analyzed for selected environmental contaminants. Twenty-four of these samples were from the the Prince William Sound resident population, four from the Gulf of Alaska transient population and, and three from the AT1 transient population. Total contaminant levels in both the AT1 and Gulf of Alaska transient populations averaged over 10 times higher than in the resident population (Table 6 Figure 13).

Resident whale samples were divided into two groups, AB pod and non-AB pod samples. Levels in AB pod were lower than in other resident whales. The non-AB pod group contained more probable first born males than the AB pod group (Table 7). Apparent first born offspring had the highest contaminant levels in the blubber. For example, of the three sibling male killer whales, AB11, AB4, and AB5, the eldest, AB5 had substantially higher total contaminant levels than the younger siblings, although AB5 was estimated to be only a few years older than AB4 (Table 8. Figure 14).

Reproductive females had the lowest levels of contaminants. The contaminant levels were substantially higher in the calves than in their mothers, for the four cow calf pairs examined. The sample included all three populations. (Table 9, Figures 15-16). Reproductive females had the lowest contaminant levels of all age and sex classes.

Discussion

Although there has been a net gain of one individual in AB pod, the changes in social structure and reduction in the number of reproductive females in the pod (Matkin et al. 1996) make it difficult to project a long-term recovery. Calves were produced by two previous mothers, AB26 and AB25. AB26 had lost her first viable calf at one year of age in 1993 and AB25 is part of the subpod that travels with AJ pod. The single mortality, AB4, was a mature male of unknown age, estimated to be at least 32 years of age and this mortality may be related to age/and or health conditions. The mean life expectancy for killer whales in Washington/British Columbia was 29.2 years (Olesuik et al. 1990).

The AB25 subpod is still traveling with AJ pod. There is no precedent for a resident pod subgroup joining another pod on an extended basis (Matkin et *al.* 1984, Bigg et *al.* 1990). When many closely related individuals within a pod die, the bonds that hold the pod together may weaken. It is conceivable that this would result in splitting of the pod and may explain while one AB pod subgroup has traveled with AJ pod for four consecutive years.

The results of our statistical analysis of resident pod associations has supported and most of our direct observations of relationships within the pods and permitted the construction of genealogical trees for the major resident pods. Details are discussed in Appendix

The fewer number of AT1 individuals observed in 1996 than in the 1995 season may in part be due to reduced field time in the spring (April) and fall (late September/ October). Only six of the eleven AT1s whales photographed in 1995 were seen in 1996. The fall winter and spring may be important periods for transient whales in the Sound and extending field seasons into those periods may increase the number of observations of transient individuals. We believe that at least 11 of the orignal 22 whales in this group are now dead, nine of these having disappeared since the EVOS in 1989. There has been no recruitment in the AT1 group since 1984. It is conceivable that this group, determined genetically distinct by mtDNA analysis, is headed for extinction.

Killer whales area use varied from year to year, although general patterns did emerge. Resident groups and the AT1 transients appeared to avoid one another. For example, Zone 1 was heavily used by AT1s and resident whales, but generally not in the same year (the difference between expected and observed use for the two groups are negatively correlated: R2 = 0.20). The adjacent Zone 2 displayed a related pattern of heavy use by resident pods and avoidance by AT1s. These two Zones comprise Knight Island passage, and are a main travel-way for whales using the west side of the Sound. Resident whales seldom were encountered in Zone 3, the southwest bays and passages, an area regularly used by transient whales hunting seals. Zone 5, including Montague Straight and waters around northern Knight Island, received more than expected use from GOA transients, while the eastern Sound (Zone 7) was avoided. The negative associations between residents and transient groups may occur because of active avoidance, or because foraging opportunities do not coincide for the two groups, one of which feeds on fish and one on marine mammals.

Barrett-Lennard et al. (1995) estimate that transient killer whales each require 73 kgs of marine mammal meat per day. Assuming that the average adult harbor seal weighs 70.7 kgs (Frost et. al. 1996), and that harbor seals comprise 24% of transient whale's diet by weight (Table 3), then each whale would consume 0.25 seals per day (more if not all

the seals were adults). Multiplied by our estimate of 562-964 transient whale days per year in the study area, this gives an estimated 140 to 241 harbor seals killed in the study area each year; or probably twice that many (280 to 481) killed throughout the Sound. The actual mortality to harbor seals was probably toward the low end of this estimate in 1985 and 1995 and towards the high end in 1988-1990. Preliminary analyses of behavioral data suggest that the frequency of offshore-foraging (done while hunting Dall's porpoises) has been increasing since 1991, while the frequency of nearshore-foraging (hunting harbor seals) has not. This suggests a possible shift towards reliance on porpoises over harbor seals (note that harbor seal kills have rarely been observed since 1993, Table 3).

This estimate of harbor seal mortality should be considered in light of Frost et al.'s (1996) harbor seal population projections. These projections showed that if the harbor seal population of 5,200 was already at carrying capacity, then an additional mortality (e.g. from killer whales) of 100-300 per year would result in a population below carrying capacity. However, if the harbor seal population of 5,200 is currently below carrying capacity then an annual additional mortality of 100-300 seals is not enough to prevent the population from growing toward carrying capacity. In either case, although such mortality might affect rates of recovery, the harbor seal population was able to sustain an additional mortality of 100-300 seals over a ten year projection without going into decline.

One of the most striking results to emerge from genetic analysis is that each group of whales sampled was monomorphic for a single mtDNA D-loop haplotype. This is strong evidence that permanent female movements between the groups is at most extremely rare. The genetically-distinguishable groups map closely onto groups previously-identified based on association patterns and/or acoustic behavior. Fish eating (resident) whales and marine mammal eating (transient) whales are clearly distinguished.

It is not surprising that the marine mammal-eating transient killer whales had contaminant levels over 10 times greater than fish-eating resident whales, since transient whales are consuming organisms a full trophic level above residents. Although AT1 transients are not thought to travel far from Prince William Sound, they have substantial loads of contaminants that have not originated in the region. These contaminants have apparently spread via food chains from other regions. Contaminant levels have varied dramatically in blubber samples from beached killer whales collected in the eastern North Pacific and could not be correlated with location (J. Calambokidis, pers. comm.). Our findings indicate that whales from sympatric populations of killer whales may have very different levels of contaminants and that contaminant levels in individual whales may be related to individuals genealogy and reproductive history. Evidence strongly suggests that contaminants are passed from mother to offspring during lactation and that recent mothers are likely to have low contaminant levels, while first born offspring have relatively high contaminant levels. Examination of changes in contaminant levels would require resampling of the individual or a very large and unbiased sample because of the variation among individuals. Identification of the population being sampled is also an important component of interpretation of environmental contaminant data.

Conclusions

AB pod had no atypical mortalities in 1996 and demonstrated a net increase of one individual. The pod currently numbers 23 whales; seven of these whales, the AB25 subpod, continue to travel with AJpod. The reduction in the number of reproductive females and social disruption within the pod makes potential for recovery of this pod in the foreseable future doubtful. All other well-documented resident pods remain stable or increasing; overall the Prince William Sound resident killer whale population appears healthy. Not all pods were completely photographed in 1996. Long-term population assessment could be jeopardized without an annual monitoring program.

We suspect that 11 of the 22 original members of the whales in the AT1 transient group are dead. There has been no recruitment within the group since 1984. There were only four encounters with this group in 1996. The factors contributing to the decline of this group and its reduced role in the Prince William Sound ecosystem are unknown, but these changes accelerated after 1989 with the death or emigration of 9 individuals. It is conceivable this population will become extinct.

Statistical analysis and direct observation were used to delineate resident pods and to develop genealogies within pods. Results paralleled those of Bigg et al. 1990 in Washington and British Columbia. Resident killer whales of both sexes appear to remain with their mothers for life in matrilineal groups. Pods are composed of one or more matrilineal groups. (detailed in Appendix).

We introduced a second measure of search effort. While boat-days has been used in the past as a measure of effort, we used the GIS to introduce a second measure, kilometers-searched. Patterns of sightings-per-unit effort generally paralleled those found when effort was calculated as boat-days. This provides an immediate confirmation that kilometers-searched is a comparable measure of effort to boat-days. However, the technique of mapping kilometers of search effort across the Sound allowed spatial patterns to be detected. For example, while transient whales appear to use all parts of the Sound with similar frequency, area use by resident whales is biased toward the large southwest passages. Estimated predation rates on harbor seals varied from year to year, but were similar to estimates in Frost et al. (1996) of the maximum sustainable additional mortality to the harbor seal population.

Resident and transient killer whales have now been separated using numerous criteria. Spatial use patterns developed by GIS analysis have supported our direct field observations that indicated residents and transients do not associate. The examination of seasonal components of the diet of resident and transient killer whales demonstrated a clear distinction between the two killer whale types (detailed in Appendix). At least during the spring, summer and fall, residents consume fish while transients prey on marine mammals. Genetic analysis has delineated two transient haplotypes and two resident haplotypes, that all use Prince William Sound. The residents and transients of Prince William Sound belong to different populations. Residents and transients diverged once. The comparison of mtDNA D-loop sequences between Prince William Sound and British Columbian killer whales indicates that the resident and transient assemblages are separate, long-standing lineages, not evolutionarily re-occurring specialist forms.

Finally, contaminant analysis has separated the two transient populations from the resident population. The average contaminant levels for transients are 10 times higher than they are for residents.

A wide variation in contaminant levels was found in individual killer whales. This was due to wide differences in contaminant levels between populations and the role of genealogy in the transmission of contaminants. Both resident and transient killer whales appeared to pass contaminants to offspring via lactation. A first born offspring is likely to have received the greatest load of contaminants from its mother. At this time it is not known whether the high contaminant levels in transient killer whales might have impacts on reproductive success for those populations.

Examination of the population structure and social structure of the killer whales using Prince William Sound using genetic and other techniques is allowing a clearer interpretation of the changes that have occurred since the *Exxon Valdez* oil spill. Feeding habit studies and GIS analysis are delineating the potential impact of killer whales on the non-recovering harbor seal population. Contaminant studies examine another potential problem in the recovery of transient killer whales and provide a baseline to assess future changes in contaminant levels in individual whales

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Tables

Year	Logs	Effort*	Logs^	Search (hrs)	Enc**
1984	129	11341	129	1015.08	69
1985	60	4453	56	367.25	48
1986	60	4680	54	392.69	34
1987	29	2057	29	176.25	22
1988	68	4316	59	342.5	27
1989	206	16183	205	1193.95	88
1990	249	19604	249	1391.06	85
1991	188	15651	188	1356.1	54
1992	136	10492	136	867.3	69
1993	79	5591	76	487.45	40
1994	87	6321	87	612.47	32
1995	125	11068	125	884.1	63
1996	92	7700	92	589.98	32
Total	1508	119456	9676		663

Contents of GIS database. Table 1:

* Kilometers of search effort by all vessels. ^ Logs for which search time is available (start or end time for some log records was missing).

**Encounters with whales.

Table 2. Summary of 1996 Killer Whale Encounters

Record	Date	Begin Location	End Location	Pods
1	07/04/96	off Pt Grace	3 mi S Pt Grace	new transients
2	07/09/96	2 mi N Pleiades	1 mi SE Mummy Bay	AE
3	07/11/96	2 mi N Sleepy Bay	1 mi SE Bishop Rock	AT60
5	07/14/96	off Pt Helen	off S end Mummy Bay	AT60
6	07/16/96	off Johnson Bay	1/2 mi W McPhearson	AB,AI
7	07/17/96	off Pt of Rks	off Johnson Bay	AB,AI,AE
8	07/18/96	1/2 mi N Sleepy	2 mi NE Needle	AB,AI
9	07/20/96	off Iktua Rk	off Gibbon Anch	AJ,AB25subpod
10	07/21/96	off L Green	Green Is	AK 2 subgrp
11	07/24/96	off Needle	bet Needle and Pt Helen	AT60
12	07/25/96	1 mi S Pt Helen	1/2 mi W Cape Elring.	AB,AI,AG,AN20
13	07/30/96	W Montague Pt.	Schooner Rk	AB
14	07/31/96	1 mi NW Needle	Marsha Bay	AT1
15	08/02/96	Pt Grace	2 mi N Sleepy Bay	AT1
16	08/02/96	2 mi S Panhat Pt	1.5 mi S Panhat	AT1
17	08/02/96	1/2 mi N Amerck	1.5 mi N Sleepy Bay	new poss. trans.
18	08/03/96	E. Latouche I.	off Hanning Bay	AE,AN10,AS,AG
4	08/03/96	S end Latouche I	off Macleod Hbr	AG
19	08/07/96	SW Little GreenI.	1/2 mi N Channel I.	AI
20	08/09/96	Needle	SE Pt Grace	AE
21	08/09/96	off Sleepy Bay	1/2 mi N Evans Pt.	AT1
22	08/12/96	W of Needle	W Cape Cleare	AI,AD
32	08/13/96	E of Hogan Bay	S end L Green Is	AI
23	08/14/96	Hanning Bay	Hanning Bay	AE
24	08/15/96	1.5 mi N Needle	2 mi S Pt Helen	AT60
25	08/17/96	1/2 mi N Pt Bazil	Port Chalmers	AE
26	08/19/96	off Pleiades	Lower Herring Bay	AI
27	08/21/96	E Fleming I.	N. of Bainbridge Psg	AE
28	08/24/96	NW Bishop Rock	S of Mummy Bay	AE
29	08/25/96	S of Mummy Bay	off Pleiades	AE
30	08/28/96	W ofHanning Bay		AN10,AI,AE,AJ,AF
31	09/02/96	off Little Bay	W of Needle	AB,AI

le 3.	Recruitment in P	rince William Sc	ound Resident Pods		{whale number(mo	thers number)}
POD	AB	Al	AK	AE	AJ	AN10
84/85	· · · · · · · · · · · · · · · · · · ·	· · · · · · · · ·	8(6)	13(11)	· · · · · · · · · · · · · · · · · · ·	
85/86	36(23),37(6)		9(2)		-	
86/87	38(31),39(25)	• •••• • • • • • •				38(10)
87/88	40(14), 41(8)			∣15(10)	26(22),27(20)	40(35)
	42(32),43(17)		·····		28(24)	+0(33)
	44(22)		· · · · · · · · · · · · · · · · · · ·			a a a
88/89			· · · · · · · · · · · · · · · · · · ·	16(2),17(5)	29(8)	41(8)
89/90			10(2)	18(11)	30(3)	
90/91	45(16)	· · · · · · · · · · · · · · · · · · ·	11(6)		100(0)	45(35)
91/92	46(25),47(32)			······	31(24),32(22)	46(10),47(11)
002				· ··· · · · · · · · · · · · · · · · ·	33(13)	
92/93	48(26)		12(7)	19(11)	34(3),35(8)	
					36(4)	
93/94	49(22)	,	13(2)		37(18),38(20)	48(8)
94/95		÷ · · · · · ·	13(2)	20(2)	37(10),30(20)	49(11)
95/96	50(26),51(25)	7(4)	· · · · · · · · · · · · · · · · · · ·	20(2)	39(13)*	50(35),51(12)
				··· ··· · · · · · ·		
	Mortalities in Pri	nce William Sou	ind Resident Pods		{by whale number	}
POD	AB	Al	AK	AE	AJ	AN10
84/85	9,15,34-			8-		
85/86	1,7,12-		5-	4-	23-	
86/87	28-	···· · · · · · · · · · · · · · · · · ·	· · · · · ·		n ∳ s s ministra de la seconda de En seconda de la seconda de	6-
87/88	6-			7-		
88/89	13,18,21,23			12-	• • • • • • • • • • • • • • • • • •	2-
	30,31,37-					
89/90	8,19,20,36					
	42,44-	i . 			a marka a series e s	
90/91	¦29-			· · · · · · · · · · · · · · · · · · ·	· · · · · · · ·	
91/92	· · · · · · · · · ·				· · · · · · · · · · · · · · · · · · ·	
92/93	an a	• • • · ·			5-	5-
93/94	2,16,38,41		· · · ·	13-	·11-	
	48-				- ··· · ·	
94/95			4-	1 1.	6-	
95/96	4-	···· · · · · · · · · · · · · · · · ·		i in Procession and Statement	**	
· · · · · · ·	*to be confirme			completely photograph		

Year	Search	Enc. whls	Radio days	Both	Presrch	Pradio	Pmissd	Add. whls	Season whls	kw/ day	Annual
1984	129	71			NA	NA	0.37	188	259	2.03	739
1985	60	74	2	1	0.92	0.15	0.07	18	92	1.59	580
1986	60	51			NA	NA	0.37	87	138	2.34	854
1987	29	19			NA	NA	0.37	40	59	2.12	775
1988	68	85			NA	NA	0.37	91	176	2.64	964
1989	110	162	15	5	0.77	0.34	0.15	89	251	2.3	839
1990	118	139	16	3	0.68	0.39	0.19	125	264	2.27	827
1991	110	43	9	1	0.56	0.5	0.22	128	171	1.58	576
1992	77	69	14	2	0.57	0.5	0.21	101	170	2.21	807
1993	67	42	1	1	NA	NA	0.37	92	134	2	729
1994	77	17	19	1	0.22	0.83	0.14	114	131	1.66	607
1995	105	61	10	2	0.71	0.36	0.18	100	161	1.54	562
1996	64	27			NA	NA	0.37	91	118	1.85	674

 Table 4: Estimation of transient whale-days in the study area

<u>Search</u>: Days on which at least one boat was searching for whales. <u>Enc. whis</u>: Transient whale-days recorded during encounters (e.g. three transients encountered on one day is three whale-days).

<u>Radio trans</u>: Number of radio reports of presumed transient killer whales (< 4 whales recorded in bays or passages). No radio logs were available for years left blank. <u>Both</u>: Number of days on which transient whales were detected both during an encounter and by a radio report.

<u>P</u>: The probability that: <u>Presrch</u>, transient whales will be detected by the researchers; <u>Pradio</u>, transient whales will be detected in a radio report; <u>Pmissd</u>, transient whales that are present will be missed by both researchers and radio reports. NA, estimate not available (no radio logs, 1984, 1986-88; <20 radio reports, 1993). Note that when Presrch and Pradio are not available, Pmissd is set equal to 0.37, the average probability that researchers will fail to detect transient whales over years when radio reports were available.

<u>Add. whis</u>: Additional killer whale days documented by radio reports and extrapolated from the calculated probability of failing to detect transients that were present. An average transient group size of 4.5 is assumed.

<u>Seas. whis</u>: The number of transient whale-days estimated for the field season (equals the sum of Enc. whis and Add. whis).

<u>kw/day</u>: Average number of killer whales using the study area per day during the field season.

<u>Annual</u>: Estimated annual transient whale-days of use in the study area (kw/day times 365 days per year.

Year	Harbor	Dall's	Harbor	Steller Sea Lion	Unknown
	Seal	Porpoise	Porpoise		
1988	0	2	0	0	1
1989	2	1	1	0	0
1990	2	3	0	0	0
1991	3	2	0	0	0
1992	1	2	· 0	0	0
1993	0	1	0	0	0
1994	0	1	0	0	0
1995	2	0	1	1	0
1996	0	2	0	0	3
All	10	14	2	1	4
Weight*	70.1	130	54	272	
kgs^	707	1820	108	272	
%**	0.24	0.63	0.04	0.09	

 Table 5: Marine mammal kills by transient killer whales, 1988-1996

* Body weight of average adult for each species, in kilograms. ^ Cumulative mass of animals killed.

**Percent of diet comprised of each species, by mass.

Contaminai CB	nt	Resident (AB pod)	(non-	Re AB Pod	esident	Trans	ient	AT1	Transi	ent GOA
congener		n = 13		n=9		n=3			n=4	
eengene.	101		730		240			6667		6125
	105		72		16			183		488
	118		300		97			923		3337
	128		100		44			1023		6422
	138		540		130			4333		6925
	153		760		270			7567		2856
	156		20		5			38		78
	157		5.2		Ő			20		38
	170		120		29			1216		1183
	1.80		370		90			3067		3925
	189		1.1		0			20		5525
			•••					20		5
			210		100	I	,	1467		1833
НСВ										1000
			4200		· 1400		6	7333		102000
p,p'-DDE							-			.02000
• • •			35		12			4733		4912
o,p'-DDD										
			300		100			2123		2483
p,p'-DDD										
			460		210			3347		3378
o,p'-DDT										
			150		32			2166		3300
p,p'-DDT										

Table 6. Average contaminant levels (in ppb) in Prince William Sound resident and transient killer whales

Whale	Sex	Age	Genealogy*
AB RESIDENTS			
AB26	F (repro)	b<1974	2 calves,AB48, AB50
AB3	Μ	b1966?	2nd? offspring of AB6
AB 40	М	b1988	calf of AB14
AB27	?	b 1974?	3rd offspring of AB34
AB11	Μ	b 1975?	3rd offspring of AB10
AB4	М	b1966?	2nd offspring of AB10
AB5	M	b<1963	1st offspring of AB10
AB17	F (repro)	b<1974	recent calf AB 43
AB35	Μ	1976?	2nd? offspring of AB17
NON-AB RESIDENTS			
AE2	F (repro)	b1975?	2 calves, AE16,AE20
AE16	Μ	b1989	1st born offspring
AE10	F(repro)	b<1973	1 calf AE15
AE15	?	b1988	1st born offspring
AE11	F (repro)	b<1970	calves AE13,AE18,AE19
AE14	М	b1976?	1st born offspring of AE8
AE1	M	b<1963	1st born offspring of AE4
AE6	Μ	b1979?	1st born offspring of AE5
AE3	Μ	b1977?	3rd born offspring of AE4
AE9	М	b<1963	1st born offspring of AE12
AN1	М	b<1963	1st born offspring of AN5
AI2	Μ	1968?	2nd born offspring of AI3
AI6	М	1975?	3rd born offspring of AI3
AS12	М		
AS 95-16	M		
AT1 TRANSIENTS			
AT18	F?	b<1974	
AT9	F	b<1970	at least one calf, AT10
AT10	?	b1980?	offspring of AT9
GOA TRANSIENTS			
AU3	F	b<1980	one known calf, AU4
AU4	?	b1995	offspring of AU3
AU2	F?		
AC2	F?		

Table 7. Life history data for whales sampled for environmental contaminants

* genealogies developed by direct observation and association analysis

Table 8.	Contaminant	levels ir	three	sibling	male
resident	killer whales			-	

Contaminant	AB11 (male b1975?)		AB4 (male b1966?)	AB5 (m: b<1963)	ale,	
CB congener			,	······		
77	7	4		3		1.8
105		5.4		10		13
118	l i i i i i i i i i i i i i i i i i i i	59		74		100
126		4		2		1.6
156		3		2		3.6
157		2		2		1.1
169		5		3		2.2
170		6		18		44
180		59		67		120
189		3		2		1.2
101		110	2	00		250
128		70		23		42
138		58	1	00		160
153		170	2	20		290
opDDD		10		4		16
ppDDD		51		61		110
ppDDE		660	7	80		1800
opDDT		71	. 1	70		250
ppDDT		10		11		47
HCB		47		57		73

RESIDENTS						TRANSIEN	TS	
Contaminant	AE2 (cow)	AE16 (calf/AE2) A	E10 (cow)	AE15 (calf/AE10)	AT9 (cow)	AT10 (calf/AT9)		4 (calf/AU3)
CB Congener								
77	2.1	1.5	1.9	1.5	1.3	1.9	8.3	3.8
105	15	230	4.2	170	120	150	130	750
118	62	940	21	810	540	930	710	4300
126	1.9	1.3	1.7	1.4	1.1	1.7	7.3	3.3
156	1.3	60	1.2	48	24	38	51	120
157	1.2	17	1.1	14	5	26	4.8	73
169	2.6	1.9	2.3	2	1.6	2.4	10	4.6
170	83	340	21	270	650	1700	820	1600
180	200	100	63	.960	1900	3500	2400	5200
189	1.4	1	1.2	. 1	11	22	5.3	2.5
101	160	2900	52	1	3800	8000	2600	17000
128	22	290	6.6	300	570	1400	540	22000
138	140	1600	45	17.00	2600	5400	3000	12000
153	310	2800	97	1	4600	9000	5100	20000
opDDT	93	2000	22	1	4300	8700	2600	16000
ppDDT	85	430	14	220	870	2000	730	2800
ppDDE	92	22000	- 190	1	38000	91000	21000	210000
opDDD	3.4	120	3	110	470	870	210	2800
ppDDD	67	980	6.7	680	1500	2700	1100	9000
HCB	110	720	25	460	1000	1000	130	3800

Table 9. Contaminant levels (in ppb) in Prince William Sound resident and transient cow calf pairs
















DLS, PWSSC 1996

Figure 2 Kilometers of search effort in1984-95



Zones of approximately even search effort for killer whales

DLS, PWSSC 1996

Figure 3.

Zones of approximately even search effort (No effort



Figure4. Resident pod encounter rate 1984-96 Arcsine transformed ANOVA p= .60 (84-95)

34



Figure 5. Total number of whales in AB pod and in all other resident pods 1984-1996



Figure 7

AT 1 Group: Individual Sighting Histories







Effort in total field days

.

encounters per 100 km search effort (light right half circles for AT group in 1984-89



Blue = km of search errort. Yellow = sightings of AT pod(s) per km effort.

Figure 3. Distribution of search effort (dark, left half-circles) and encounters per 100 km search effort (light right half circles for AT group in 1990-95



Blue = km of search effort. Yellow = sightings of AT pod(s) per km effort.

DLS, PWSSC 1996

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¹⁹⁹⁶ Blue = km of search effort. Yellow = sightings of AT pod(s) per km effort.

Figure **S**c. Distribution of search effort (dark, left half-circles) and encounters per 100 km search effort (light right half circles for AT group in 1996

DLS, PWSSC 1996

Figure 9.a. Distribution of search effort (dark, left half-circles) and encounters per 100 km search effort (light right half circles for GOA transient groups in 1984-89.



DLS, PWSSC 1996

Blue = km of search effort. Yellow = sightings of AC AU AT30 AT50 AT60 AT70 AT? pod(s) per km effort.

Figure **9b.** Distribution of search effort (dark, left half-circles) and encounters per 100 km search effort (light right half circles for GOA transient groups in 1990-95.



Blue = km of search effort. Yellow = sightings of AC AU AT30 AT50 AT60 AT70 AT? pod(s) per km effort.



1996 Blue = km of search effort. Yellow = sightings of AC AU AT30 AT50 AT60 AT70 AT? pod(s) per km effort.

DLS, PWSSC 1996

Figure **9***c*. Distribution of search effort (dark, left half-circles) and encounters per 100 km search effort (light right half circles) for GOA transient groups in 1996.

Figure 10a. Distribution of search effort (dark, left half-circles) and encounters per 100 km search effort (light right half circles) for resident pods 1984-1989.



Blue = km of search effort. Yellow = sightings of Resident pod(s) per km effort.

DLS, PWSSC 1996

Figure 10b. Distribution of search effort (dark, left half-circles) and encounters per 100 km search effort (light right half circles) for resident pods in 1990-95.



Blue = km of search effort. Yellow = sightings of Resident pod(s) per km effort.

DLS, PWSSC 1996



1996 Blue = km of search effort. Yellow = sightings of Resident pod(s) per km effort.

DLS, PWSSC 1996

Figure 10c. D encounters per pods in 1996.

Distribution of search effort (dark, left half-circles) and r 100 km search effort (light right half circles) for resident





TRENCZON.XLS

Figure 12. Encounter rates with AT1 and GOA transient groups and resident pods by zones, 1984-1996



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D. Scheel 1/9/97 3:01 PM









Figure 14

Figure 15

Contaminants in AT1 and GOA Transient Killer Whale Cow/Calf pairs in Prince William Sound, Alaska





Contaminants in Resident Killer Whale Cow/Calf Pairs

Whale

Figure 16





Appendix1

Data Dictionary

Data dictionary, Long-term killer whale database (NGOS & PWSSC/OSRI)

	_		Stored	Display				
	Source		Width	Width	Туре		Description	Units
							, each log has a vessel path attatched to it.	
_ •	Log	meta	6			-	Year-page number of log (numbered consecutively)	
		primary	8	-	3 Date	-	Day, month, year of vessel log (repeated on Encounter)	Date
	Log	meta	12		2 Char	-	Name of vessel (repeated on Encounter)	- 1
	Log	meta	16		6 Char	-	Initials of personnel on vessel	-
	Log	primary	50		5 Char	-	Place name where vessel began the day	-
	Log	primary	50		6 Char	-	Place name where vessel ended the day	-
	Log	primary	6		S Num	2	Begining time of log	dec hours
	Log	primary	6		S Num .	2	End time of log	dec hours
	Log	primary.	6	(S Num	2	Duration spent searching	dec hours
	Log	derived	6	(S Num	2	Duration spent with whales	dec hours
LS-length	Log	derived	5	1 1	5 Num	1	Length of trackline surveyed, as recorded on the Log	dec miles
	Log	primary	200		5 Char	-	Running commentary on events *	-
	NB: File n	ame LOG	94.WEA	Up to three	e weath	er record	ds may be recorded per log	
Log-ID	Log	meta	6	i (6 Char	-	Year-page number of log (e.g. 94-001, 94-002)	
Wea-time	Log	primary	e	i (3 Num	2	Time of weather/sea state observation	dec hours
Wea-cov	Log	primary	4		4 int	0	Cloud cover as percent	-
Wea-wind	Log	primary	7	•	7 Char	-	Wind as: SW15, NE40 etc (direction and velocity in knots)	-
Wea-vis	Log	primary	3	: :	3 Int	-	2 char code for visibility (see list)	
Wea-sea	Log	primary	3	: ;	3 Int	-	2 char code for sea state (Beaufort)	Unk
Wea-prec	Log	primary	7	, .	7 Char	-	Precipitation - none, fog, Itrain, modrain, hvrain, snow	
Wea-comm	Log	primary	75	7	5 Char	-	Location of weather observation, commentary on weather	
	NB: File n	ame LOC	694.SIGH	T. Multiple	whale si	ightings	may be recorded per log.	
Log-ID	Log	meta	e	; 1	5 Char	-	Year-page number of log (e.g. 94-001, 94-002)	
Enc-ID	Log	meta	7	· ·	7 Char	-	Encounter number in database (94-001a, 94-002a, 94-002b,)	-
LWhl-time	Log	primary	6	;	6 Num	2	Time of whale observation	dec hours
LWhl-spp	Log	primary	4	Ļ ،	4 Char	-	2-3 char code for species observed (not all porpoise sightings noted)	-
LWhi-loc	Log	primary	50) 1.	5 Char	-	Place name where sighting occured *	-
LWhl-no	Log	primary	3	3	3 Int	-	Number of whales recorded in the sighting in the Log (porpoise and HW	animals
LWhi-behv	Log	primary	7		7 Char	-	See list	-
LWhi-comm	Log	primary	75	5 7	5 Char	-	Commentary on whale observation w/pods present if KWs	

Data dictionary, Long-term killer whale database (NGOS & PWSSC/OSRI)

	_			Display		_		
ltem	Source			Vidth		Dec.	Description	Units
							sheets per log; each sheet has a vessel path attatched to it.	
Log-ID	Log	meta	6		Char	-	Year-page number of log (e.g. 94-001, 94-002)	-
Enc-ID	Log	meta	7		' Char	-	Encounter number in database (94-001a, 94-002a, 94-002b,)	
Enc-date	enc	primary	8	-	3 Date	-	Day, month, year of animal encounter (repeated on Log)	Date
Enc-platform	enc	meta	12		2 Char	-	Name of the vessel (repeated on Log)	-
Observers	enc	meta	16		6 Char	· -	Initials of observers making encounter record	-
EB-time	enc	primary	6		3 Num	2 2	Begining time of encounter	dec hours
EE-time	enc	primary	6	e	6 Num	2	End time of encounter	dec hours
EB-Loc	enc	primary	50	15	5 Char	-	Place name where encounter began *	-
EE-Loc	enc	primary	50	15	5 Char	-	Place name where encounter ended *	-
Pods	enc	derived	16	. 8	3 Char	-	2-3 char codes for pods represented at encounter *	-
Mi-trav	enc	derived	5	5	5 Num	1	Nautical miles traveled with pod, as recorded on form	dec miles
Tot-whl	enc	primary	3	3	3 int	-	Total number of whales counted in the encounter (field estimate).	whales
Conf-whl	photos	derived	3	3	3 Int	-	The best estimate of the number of whales at the encounter	whales
Conf-type	photos	meta	5	Ę	5 Char		MajR, major resident pod (AB, AI, AE, AK, AJ, AN10, AN20) using	
							pod size for that year; Photo, number of whales actually	
							photographed; Field, Field estimate	
adM	enc	primary	3	:	3 Int	-	Total number of adult males counted in the encounter (field estimate)	whales
adF-I	enc	primary	3	;	3 Int	-	Total number of adult females or immatures counted (field estimate)	whales
juv-calf	enc	primary	3	;	3 Int	-	Total number of juv/immatures counted in the encounter (field estimate)	whales
Recg-ind	enc	primary	200	10	6 Char	-	3-4 char names of individuals recognized (for individuals	
U U							photographically documented, see G. Ellis database). For transient	
							whales, whales photographically documented. *	-
Tot-pho	enc	primary	3	-	3 Int	-	Total number of whales photographed (field estimate). For transient	
		, ,					whales, number of whales photographed (from photographic	
							database).	whales
Tot-har	enc	primary	3		3 Int	-	Total number of whales harassed by researchers	whales
Oil	enc	primary	32		8 Char	-	None if no oil present, otherwise type of oil present	-
Enc-Dur	enc	derived	6		6 Num	2	Encounter duration (EE-Time - EB-Time)	hours
2.10 20.			4.FILM N	May be m	ultiple i	rolls of f	ilm per encounter sheet	
Log-ID	Log	meta	6		6 Char		Year-page number of log (e.g. 94-001, 94-002)	-
Enc-ID	enc	meta	7		7 Char		Encounter number in database (94-001a, 94-002a, 94-002b,)	
Film-date	enc	primary	8		8 Date		Day, month, year of film roll	Date
Film-roli	enc	primary	3		3 Int	-	Number of film rolls taken	-
Film-ini	enc	primary	8		8 Char	_	Initials of photographer	
1 000-00							g tapes per encounter sheet.	
		meta	6		6 Char		Year-page number of log (e.g. 94-001, 94-002)	
Log-ID	log	meta	7		7 Char		Encounter number in database (94-001a, 94-002a, 94-002b,)	
Enc-ID	enc		3		3 Int		Tape number ID of recording	-
Rec-tape	enc	primary	3 1		1 Char		Side on which tape was recorded (A or B)	-
Rec-side	enc	primary	1		i Ghai	•	Side on which tape was recorded (n or b)	

Rec-beg	enc	primary	4	4 Int	-	Counter number where recording began	-
Rec-end	enc	primary	4	4 Int	-	Counter number where recording ended	-
	NB: File	e name ENC94	ACTIVE M	ay be multiple	e acti	vites per encounter sheet.	
Log-ID	log	meta	6	76 Char	-	Year-page number of log (e.g. 94-001, 94-002)	
Enc-ID	enc	meta	7	7 Char	-	Year-page number-encounter	
AB-time	enc	primary	6	6 Num	2	Beginning time of activity	Time
AE-time	enc	primary	6	6 Num	2	Ending time of activity	
Activ-behv	enc	primary	14	14 Char	-	See list	-
Activ-vess	enc	primary	50	15 Char	-	Vessel traffic	* -
Activ-inter	enc	primary	50	15 Char	-	Interaction with whales by other boats	* _
Activ-grp	enc	primary	50	15 Char	-	Sub-groupings of whales listing individual IDs	* _
Activ-note	enc	primary	50	15 Char	-	Additional information on the activities	* _
	NB: File	e name ENC94	BIOP May	be multiple p	ods b	iopsied per encounter sheet	
Log-ID	log	meta	6	76 Char	-	Year-page number of log (e.g. 94-001, 94-002)	· -
Enc-ID	enc	meta	7	7 Char	-	Year-page number-encounter	-
Biop-pod	enc	primary	32	8 Char	-	2-3 char codes for pod biopsied	-
Biop-ID	enc	primary	4	4 Num	-	1-2 numerical code for identity of whales biopsied	. -

Appendix 2

Association Patterns and Genealogies of Resident Killer Whales (Orcinus orca) in Prince William Sound

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Association Patterns and Genealogies of Resident Killer Whales (Orcinus orca) in Prince William Sound, Alaska

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1. INTRODUCTION

It has been well-established that individual killer whales can be recognized by unique marks, scars and pigmentation patterns. During the past 25 years a number of studies have used this attribute investigate the life history, behavior, population dynamics, vocalizations, abundance and movements of killer whales in coastal waters of the Eastern North Pacific including Prince William Sound, Alaska (Balcomb et *al.* 1982, Bigg et *al.* 1990, Ford 1991, Olesiuk et *al.* 1990, Matkin et *al.* 1992.)

As a result of these studies, at least two forms of killer whale, termed "resident" (fish eating) and "transient" (marine mammal eating), have been identified in the nearshore waters of Puget Sound, British Columbia, and in southern Alaska (Bigg 1982, Leatherwood et *al.* 1984, Bigg et *al.* 1990, Leatherwood et *al.* 1990, Matkin et *al.* 1994). Numerous differences between residents and transients have been described, including dietary specializations (Bigg et *al.* 1987, Morton 1990, Saulitis 1993, Saulitis *et al.* in prep.), acoustical differences (Ford 1991) and genetic variation (Stevens et *al.* 1989, Hoezel and Dover 1990, Barrett-Lennard et *al.* in prep). Resident and transient killer whales have not been seen traveling together (Morton 1990, Matkin *et al.* 1994, Saulitis 1993) Resident whales remain in their pods for life, while transients appear to have a more fluid social structure (Bigg et *al.* 1990).

Life history parameters for resident killer whales in British Columbia and Washington State were developed by Olesiuk et *.al* 1990. Females in that study had a mean life expectancy of 50.2 years. They typically gave birth to their first viable calf (a calf that survived to age 0.5 yrs) at 14.9 years of age and produced an average of 5.35 viable calves over a 25.2 year reproductive lifespan. Their maximum longetivity was about 80-90 years. Neonate mortality was estimated at 43%. Males had a mean life expectacy of 29.2 years, typically attained sexual maturity at 15.0 years and physical maturity at 21.0 years of age, and had a maximum longetivity of about 50-60 years. The dorsal fin of males began growing at the age of sexual maturity and attained a height to width ratio of 1.6-1.8 by about 21 years.

The direct observations and analysis presented here are modeled after the field. methods and analysis developed by Bigg et al. (1990) to study the social organization of resident killer whales in the coastal waters of British Columbia and Washington State. They determined the membership of social groups by observing which individuals traveled most frequently together and by examining the relative strength of bonds among individuals and among groups. The strength of bonds was established by direct observation of the proximity of whales to one another and from an analysis of the association of individuals in photographic sequences. Individuals that consistently surfaced within 1-2 body lengths (5-10m) of each other were considered to be the most strongly bonded, whereas individuals within a social group that rarely surfaced near one another were considered to be the most weekly bonded. They described pods as a group of individuals that travels together the majority of the time. Intrapod groups were found to consist of a cohesive group of individuals that always traveled in close proximity. They constructed genealogical trees from known genalogies and from inferrences about genealogy based on the strength and continuity of bonds among pod members. The genealogical trees indicated intrapod groups were matrilineal groups. A typical matirilineal groups consisted of 2-3 generations and were considered the basic unit of social organization. Pods appeared to be comprised of related matrilineal groups.

Systematic observation and photography of killer whales in Prince William Sound began in 1983. Resident pods were first delineated by direct observation in 1984 (Ellis 1984) and described in Leatherwood et. *al.* (1990). The most recent pod affiliations determined by direct observation of Prince William Sound resident killer whales were described by Heise et. *al.* (1992). The current study uses direct observations in conjunction with an index of the degree of association among individuals in photographic sequences to examine the social organization and genealogy of resident killer whales in Prince William Sound, Alaska.

2. Methods

Summary

After Bigg *et al.* (1990), the identity and individual membership of each pod and the structure within pods was determined by observing which individuals travelled together most frequently and by examining the relative strength of bonds among individuals within the groups. The relative strength of bonds was determined by (1) direct observation of the proximity of individuals to one another as seen during field observations and in photographs; and (2) and index of the degree of association among individuals in photographic sequences. This index was based on photographs collected 1984-1995. Pod affiliation of individuals and intrapod structure was illustrated by the construction of dendrograms. A listing is provided of each individual by pod affiliation, using their name code and when known, their sex, year of birth, year of death, and their mother's identity. When birth year was uncertain, ages were estimated. Maternal genealogical trees were constructed based on known mother/offspring genealogies inferred from the strength and continuity of bonds between individuals.

Field Methodology

Field observations and photographs of a single community of resident killer whales were collected in Prince William Sound, Alaska and adjacent waters from 1983 to 1996. Photographs were taken throughout the Sound, however the study was centered in southwestern portion. Most of the observations made and photographs taken were in Montague Strait and Knight Island Passage. Fieldwork occurred during the months from April to October. The greatest effort and majority of encounters with resident pods occurred in July, August and September. A number of vessels from 4-9m in length were used to approach the whales and obtain photographs. The vessels ranged in length from 4m to 9m and in power from 50hp outboard motors to 185hp deisel inboard/outboard engines.

Whales were located by non-random searches of the Sound based on current and historical sighting information. A network of small vessels supplied sighting reports on VHF radio. Whales were located visually or by listening for killer whale calls with directional hydrophones.

During each encounter individual whales were typically photographed several times from a distance of 15-30m. Individual identification photographs were taken of the port side of each whale, showing details of the dorsal fin and white saddle patch. An effort was made to move systematically through the pod and obtain lateral view photographs of individuals that filled at least 50% of the frame. Whales were followed until all whales were photographed or until weather and/or darkness made photography impracticable.

In the early years of the study we used a 35mm SLR Nikon FM2 camera with 300mm telephoto lens and autowinder mounted on a shoulder brace. Later this setup was replaced by a 35mm SLR Nikon 8008 autofocus camera with shoulder brace. Ilford HP5 film was exposed and processed at ISO 1600.

Specifics of each encounter with killer whales were recorded including date, time, duration, and location of the encounter. Also recorded were the total number of individuals present, the identity of the individuals that could be immediately recognized, and the general behavior of the whales (i.e. feeding, resting, traveling, socializing, and milling).

Analysis of Photographs

Throughout the study, each photographic frame was examined numerous times with a disecting microscope to ensure that all individuals had been correctly identified (Bigg*et al.* 1987). The identity of known mother-offspring pairs was also noted. The determination of mothers of new calves was based on the consistent close association of calves with a female. Identified individuals were recorded in a standard format for computer input. Uncertain identifications were not included in the analysis.

Each whale was assigned an alpha-numeric code which was based on the system of Leatherwood *et al.* (1984) and catalogue by Heise *et al.* (1992). The first letter in the code was "A" to designate Alaska, followed by a letter (A--Z) indicting the pod. Individuals within the pod received sequential numbers. Pods that resulted from a

splitting of a pod shared letter designations, ie AN10 and AN20 pod. These pods were named after a distinctive matriarch within the pod.

Sexing and Aging of Individuals

In order to establish genealogies, sexes and estimated ages were determined. Sexually mature males were differentiated from females and immature males by the dorsal fin height to width ratio (HWR) which typically exceeds 1.4 by 15 years of age. Mature females were identified when they gave birth and were accompanied by a new calf. The sex of most juveniles could not be determined except in cases where the penis or the unique pigmentation pattern of the genital region was observed (Bigg *et al.*, 1989).

Actual ages could be determined for whales born during the study. The age of whales that were immature at the begining of the study was estimated when they were first seen, based on the relative size of the whale, maturation of the saddle patch, and size of the dorsal fin. The birth year for whales that matured during the study was estimated by subtracting the mean age of maturity (15 years for both sexes) from the year they matured. Females were considered to have matured in the year they gave birth to their first viable calf and males in the year in which their dorsal fin attained an HWR of 1.4 (Bigg et. al. 1990). Males that were physically mature and had dorsal fin HWR of 1.6-1.8 at the beginning of the study were considered at least 21 years at that time. The year of birth of males that were sexually but not physically mature at the start of the study was estimated by subtracting the mean age of phyical maturity from the year their dorsal fin attained HWR of physical maturity. The age of females that were mature at the beginning of the study was estimated by subtracting 15 years from the estimated year of birth of her eldest offspring. This was a mimimum estimate since her elder offspring may have died before the start of the study. Females that had not given birth for a decade or more were considered as likely post-reproductive (Olesiuk et al. 1990).

Data Analysis

Photographs were sorted into the order in which they were taken. The identity of all individuals in each frame or, optionally in the +/-1 or +/-2 adjacent frames was tallied for each encounter. Photographic frames or sequences that contained less than two individuals were deleted because they provided no information on association. Data from the remaining frames were accumulated in a 2 x 2 contingency table for each pair of whales for all years in which both individuals were photographed.

Table 1. Contingency table for organization of association data

			First Individual	
		present	absent	total
Second	present	a	b	a + b
Individua	absent	c	d	c + d
	total	a + c	b + d	n

where a + c and a + b denote the total number of occurrences of the first and second individuals respectively, a the number of joint occurrences and d the total number of frames in which neither whale occurred in years in which both were photographed.

The degree of ascoiation between individuals was measured using Coles (Cole 1949 after Bigg et *al.* 1990) association index (CAI):

$$CAI = \frac{ad-bc}{(a+b)(b+d)} \quad \text{for } ad \ge bc$$

 $CAI = \frac{ad-bc}{(a+b)(a+c)} \quad \text{ for } bc > ad \text{ and } d \ge a$

$$CAI = \frac{ad - bc}{(b + d)(c + d)} \quad \text{for } bc > ad \text{ and } a > d$$

The index was expressed as a percent ranging from + 100 to -100. One hundred per cent indicated that the joint number of occurances of each whale equaled the number of occurances of the least photographed individual, zero percent indicated that individuals were randomly distributed and a value of -100 indicated the individuals were never photographed together.

The CAI was a measure of complete association (versus absolute association) indicating values of +100 occur only when the joint number of occurances equals the number of occurances of the less frequently photographed individual. An index of complete association was used in analysis of association patterns between individuals because not all individuals were equally identifiable. For example, cows and calves always traveled to together, but young calves are not always well marked and may not be identifiable in all pictures.

Individuals and pods that were photographed very infrequently were not included in the database used in association analysis. Because of the volume of the tabulations only the CAI values for all years data \pm one frame is presented here.

Social groupings were identified from dendrograms constructed using an agglomerative average single-link algorithm (Johnson, 1967 after Bigg *et al.*, 1990) In this proceedure the CAI values among all possible pairs of individuals were compared and the pair with the highest CAI linked. Next the pair of unlinked individuals with the highest CAI were linked, or an unlinked individual with a higher mean CAI value with previously linked individuals was linked to that pair, and so on until the mean CAI dropped to 20%.

The degree of absolute association between the groups linked at $\geq 20\%$ CAI was measured using the point correlation coefficient (PCC):

PCC = square root of
$$[(a + b)(a + c)(b + d)(c + d)]$$

where a represents the number of photographs containing one or more members of both groups, b and c the number containing members of only one of the groups, and d the number containing no members of either group. The PCC index was expressed as a percent from -100 to +100 with 0 indicating random association.

The PCC index was a measure absolute association between groups that were established by the CAI. When associations among intrapod groups linked at $\geq 20\%$ CAI were compared (versus the initial comparison of associations among individuals) a switch was made from a measure of complete association to this measure of absolute association. Unlike individuals, intrapod groups were essentially equivalent in their identifiability because all contained at least some individuals that were easily identifiable. If an intrapod group was photographed more often than another group it indicated that it was traveling independently of the other.

Dendrograms were constructed using PCC values generated among groups and an agglomerative average single-link algorithm that joined these intrapod groups in the manner described above for joining individuals using CAI values. Linkages with a positive PCC association value (PCC > 0) were corroborated by observational data were designated as pods or subpods. Subpods were designated when PCC values greater than zero were only determined at the subpod level but direct observation indicated the designated subpods nearly always traveled together as a single pod.

After pods were determined using PCC values and observational data, CAI values were calculated for the individuals within each pod for all years of the study and displayed in a matrix for all pairings of individuals in the pod. These values were used in conjunction with sex, age and observational data for each individual to construct genealogical trees within the pod. Values for CAI were also calculated for the years 1984-1988 and 1989-1995 for females that matured and produced calves during the study and their apparent mothers. These values were used to examine changes in bond strength between age of males and the bond strength with their mothers was also examined.

Construction of Genealogical Trees

Both direct observation in the field and statistical analysis of association patterns was used to determine membership of groups and construct genealogical trees. Each method served as a check on the other, and provided unique information. Direct observation was most important for individuals and groups that were less frequently photographed. During group resting behavior, when whales were most tightly associated, affiliations and bond strengths between individuals were most apparent. Photographs taken at these times often contained more than one individual were particularly valuable in determing group bonds.

Maternal genealogical trees were constructed using the three basic steps described in Bigg *et al.* (1990). Possible offspring to be incorporated into the tree were selected, beginning with those born during the study, followed by those that were juvenile at the start of the study, and finally by those that were mature at the start of the study. Age of individuals increases from right to left moving across one level of a tree. Second, the potential mothers of the offspring were identified. All mature females in the offsprings pod were considered providing that they could have been at least 15 years (mean age of maturity) older than the offspring. An offsprings own mature daughters were excluded as potential mothers. Also excluded were females that matured during the study after a particular offspring was born which excluded young adult sisters as potential mothers. Third, the relative strength of bonds as demonstrated by CAI values between offspring and all potential mothers were examined. The potential mother with which the offspring was most closely bonded was assumed to be its mother. An offspring not strongly bonded to any potential mother was not assigned a mother. Matrices were cross-checked to insure that mother-offspring assignments created sibling groups that demonstrated reasonable linkage by CAI values. Genealogical trees developed statistically were checked with proposed geneologies developed from field observation and visual examination of photographs.

3. Results

A total of 2444 hours of direct observation of whales logged from 1984 to 1995. A total of 36,009 frames of film were suitable for use in statistical analysis of association patterns (Table 2).

Table 2.

The number of frames of film by year usable for analysis of association patterns

1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	Total
6,076	1,284	2,967	1,326	1,400	3,549	5,940	3,701	3,641	2,333	1,662	2,130	36,009

A total of 202 whales photographed between 1984-1995 were grouped by observation and association analysis into 9 pods (Table 3). The individuals were placed in 39 maternal genealogical trees. An additional 4 calves were observed in 1996 and placed in the genealogical trees based only on field observations. Another 158 whales were tentatively grouped into 5 pods (pod AX, 54 whales; pod AY, 11 whales; pod AS, 17 whales; pod AF, 48 whales; pod AG, 28 whales), but not included in the analysis because of insufficient field observations and photographs. The individuals in the tables and figures represent the cumulative memberships over all years of the study. In all pods and most groups, the numbers of individuals varied, members died or were born during the study.

Table 3.Pods and individuals used in analysis

Pod	Cumulative Membership*
AB	AB1-AB51
AI	AI1-AI7
AJ	AJ1-AJ38
AE	AE1-AE-20
AK	AK1-AK14
AN10	AN1-AN3, AN5-AN12, AN35,AN38, AN40-AN41, AN45-AN51
AN20	AN4, AN13-AN34, AN36-AN37, AN39, AN42-AN44
AD5	AD1-AD12, AD19, AD21-AD27
AD16	AD13-18, AD20
AN10 AN20 AD5	AN1-AN3, AN5-AN12, AN35,AN38, AN40-AN41, AN45-AN51 AN4, AN13-AN34, AN36-AN37, AN39, AN42-AN44 AD1-AD12, AD19,AD21-AD27

The intrapod groups delineated both by observation and CAI linkage almost always traveled together. In nearly all cases, the members of each intrapod group linked at $\geq 20\%$ CAI reflected groupings established by direct observations (Figures 1-7 CAI dendrogram). Most intrapod groups were centered around a reproductive female or a suspected post-reproductive female. However, in four cases single males (AE14, J02, N19, and D01) were not joined to other intrapod groups (CAI $\geq 20\%$) and in two cases pairs of males (AD02 and AD12, and AB02 and AB29) were not linked to other intrapod groups (CAI $\geq 20\%$). The nine pods examined contained 48 intrapod groups (Figure 1-7), inclusive of the male singles and pairs. With the exception of AB pod, pods were comprised of 1-9 intrapod groups (mean 4.5). Two pods (AI and AD16) contained only one intrapod group. The three subpods of AB pod contained a total of 12 intrapod groups. Intrapod groups were composed of 1-9 individuals (mean 4.2).

Most pods established by direct field observations formed a distinct cluster in the point correlation coefficient generated dendrogram linking intrapod groups at a PCC value greater than zero. (Figure 8 PCC dendrogram.) There were two exceptions. First, the AB10 subpod and AI pod were joined in the dendrogram at the PCC = 4 level. Second, AK pod was joined with AD16 pod at the PCC = 8 level. By direct observation, AN10 and AN20 pod were considered a single pod (AN pod) until 1992. After that time they were not encountered traveling together and were considered separate pods. Statistical analysis supported their designation as separate pods. AD pod was considered a single pod early in the study based on a few encounters. It is now designated as two pods (AD5 and AD16 pods) based both on long-term observational data and results of the statistical analysis.

Based on lack of PCC linkage above zero, AB pod was divided into three subpods (AB10, AB17, AB25), although prior to 1994 direct field observations indicated they very rarely traveled separately. Since 1994 the AB25 subpod has split off and travels with AJ pod. No other subpods were determined in the study.

Genealogical trees were constructed by first establishing all of the known mother/offspring relationships. There were 58 offspring born during the study that appeared in the photographs used for association analysis. In all but two cases (AB41 and mother AB8, and AK14 and mother AK6), the CAI value between mother and known offspring was higher than for any other pairings of individuals. Direct observation also indicated known offspring maintained their strongest bonds with their mothers. Three offspring born at the beginning of the study were still most strongly bonded to their mothers after 12 years. These were mother/offspring pairs AI3/AI4 (CAI 27), AK6/AK8 (CAI 34), and AE11/AE13 (CAI 46).

The strength of the bond between females and their mothers declined in the nine cases where females first became reproductive during the study and their mother remained alive throughout the study (Table 4)
Reproductive offspring	Year of First Calf	Mother	CAI 1984-1988	CAI 1989-1995
AJ3	1990	AJ8	28	13
AJ4	1994	AJ8	57	20
AJ13	1992	AJ14	40	33
AK7	1993	AK6	51	24
AN10	1987	AN9	22	-20
AN11	1992	AN9	30	23
AN26	1990	AN23	53	34
AN31	1990	AN20	28	19
AN35	1988	AN9	29	2

 Table 4.
 Coles Association Index (CAI) values for mothers and female offspring that produced their first calf in 1987 or later

There were 31 juveniles (age estimated 10 years or less in 1984) at the beginning of the study. All but 5 of these whales remained most closely bonded to the whale that by direct observation appeared to be their mother. Three of these exceptions were females that produced calves (AK7, AN8, AN11) and were then most closely bonded to their calves. The strongest adult bond for these three whales was with their apparent mother. One juvenile male, AN19, had a stronger bond with apparent sibling AN18 (CAI 16) than with his apparent mother AN17 (CAI 4). The juvenile AB18 had a stronger bond (CAI 58) with a young calf, AB41, in his intrapod group than with his apparent mother AB7 (CAI 22).

The strength of bonds between male offspring and their mothers varied considerably (Table 5). There was no clear relationship between age of the whale and the CAI value with its mother (Figure 9).

Table 5. Coles Association Index (CAI) values for male killer whales and their mothers

Whale	Estimated Age*	CAI Value	Whale	Estimated Age*	CAI Value
AB1	33	25	AJ9	18	32
AB3	30	20	AJ16	27	48
AB24	25	39	AJ17	29	18
AB35	19	35	AJ21	19	31
AB40	7	59	AJ25	22	48
AD3	27	50	AK1	27	20 . 34
AD4	27	6	AK4	27	29
AD6	11	68	AN1	32	66
AE1	32	25	AN3	32	31
AE3	18	21	AN7	24	17
AE6	16	38	AN14	19	47
AE14	19	24	AN21	25	54
AE9	32	31	AN24	15	55
AJ1	31	39	AN25	24	28
AJ2	27	21	AN30	16	54
AJ7	19	55	AN33	23	41

* ages estimated as described in methods, ages over 20 years are mimimum ages



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20

Estimated Age (years)

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25

30

35

Figure 9. Relationship of age and CAI value with mother for male resident killer whales its

Of the 45 maternal lineages identified in the genealogical trees (Figures 10-18) 25 were two generations, 16 were three generations and 4 were four generations. In all but one of the 4 generational trees, the oldest matriarch died during the study.

15

10

5

30

20

10

0

0

We identified 5 intrapod groups (matrilineal groups) that appeared to be destined to die out. These were the AB10 subpod (Figure 11) which consisted of the apparently post-reproductive AB10 and 3 adult male sons, AB4, AB5, and AB11; the single adult male, AB3 (Figure 12); the orphan juvenile AB45 (Figure 12); apparently postreproductive AJ12 and her adult male son, AJ16 (Figure 13); apparently postreproductive AN 34 and her adult male son, AN21 (Figure 16). Of the remaining matrilineal groups three had produced two reproductive females in one generation, and were growing and eight had produced one reproductive female and were stable. The fate of the other identified matrilineal groups will be determined as offspring born during the study mature and their reproductive potential is realized.

5. Discussion

In Prince William Sound and adjacent waters the resident groups of killer whales exhibited the same basic social organization as those in the nearshore waters of British Columbia and Washington State (Bigg *et al.*, 1990). Intrapod groups were readily identified from direct observation as well as through association analysis. We used a higher association percentage (CAI 20%) than Bigg *et al.* (CAI 15%) in establishing the cutoff level for membership in intrapod groups based on the initial Cole's association index. This decision was based on the goodness of fit of the analytical results with direct observations. The strength of the bonds among individuals appeared directly correlated with the degree of relatedness There was no immigration or emigration from these natal groups over the course of our study.

Both statistical analysis and direct observation indicated that intrapod groups associated in pods in Prince William Sound as was found also in British Columbia and Washington State (Bigg *et al.*, 1990). Pod membership was also supported by pod specific dialects in all these all these areas (Bigg *et al.*, 1990, Ford 1991, J. Ford, pers. comm.) A pod was defined by Bigg *et al.* (1990) as a group of individuals that traveled together at least 50% of the time. All of the resident pods described in Prince William Sound fit that definition. We witnessed the splitting of one pod (AN pod) during the course of the study. This was not a gradual occurrence as suggested by Bigg *et al.*. (1990) for A04, C01 and D01 pods in British Columbia, but the sudden splitting of AN pod in 1992 and the formation of AN20 and AN10 pods. The pod split along matrilines, all matrilineal groups remained intact. AN pod numbered thirty-five whales at the time it split and was the largest pod in the study at that time. A critical size may exist at which it becomes advantageous to split into two pods. AJ pod currently has grown to 35 whales and may be reaching a size where a split might occur.

There were some statistical linkages between pods that were not supported by direct observations. AD16 pod was found to be linked to AK pod (PCC = 8). This was not supported by direct observations and was apparently an artifact of small sample size. AD16 pod was infrequently photographed and was often part of multipod groups that included AK pod. AI pod was linked with AB10 subpod by (PCC = 8) as it frequently traveled with AB pod early in the study. We suspect that AI pod (7 whales in 1996) was in the final stages of a more gradual splitting with the then 35 member AB pod when the study began in 1984. AI pod traveled more independently from AB pod over the years. The pod specific dialects for AI pod and AB pod are very similar (J. Ford, pers comm.) The preponderance of males in AI pod (4 out of 7 whales in 1996) may have contributed to the initial independence of this matrilineal group. Bigg *et al.*. 1990 found that matrilineal groups with a high percentage of males tend to travel more independently. The AB10 subpod, in which 3 out of 4 members are adult males, often travels a distance away from the rest of AB pod.

Bigg *et al.* (1990) defined subpods as fragments of pods that traveled separately for some period of time. We expanded this definition to include groups that traveled together but rarely mixed. This situation occurred only for AB pod, which was divided into three subroups. By direct observation, AB pod nearly always traveled as a unit, however, in the dendrogram linking intrapod groups (Figure 8) it appeared be three separate pods (PCC >0). This indicated that although they were traveling together, the

subpods tended not to mix. These subpods were often apparent during direct observation.

Communities were described by Bigg *et al.*. (1990) as closed populations of pods that associate with one another . They described two communities of resident killer whales (northern and southern residents) with a division in range about mid- Vancouver Island, British Columbia. We found no separation of pods into communities in our area, although our study discerned matrilineal (intra-pod) groups, subpods, and pods. Resident whales from AF and AG pods photographed regularly in southeastern Alaska were observed swimming with the pods described in this study (Matkin *et al.*, 1997). One of pods described in this paper, AD pod, was photographed in Kodiak Island waters. There appear to be no community boundaries for resident killer whales from southeastern Alaska through Kodiak Island.

There were two cases where statistical analysis indicated offspring born during the study did not maintain their strongest bond with their mother. In the first case the mother AB8 died at the time of the *Exxon Valdez* oil spill and left her year old offspring, AB41 (born 1988). AB41 was more closely linked (CAI 58) to its mother's apparent sibling AB18 than to mother AB8 (CAI 50). Both the mother AB8 and sibling AB18 died following the spill, the calf AB41 died several years later (1993-4). In the second case, AK 14 was more closely linked to sibling, AK12, than to its mother, AK6. The problem was sample size as only a few photographs of AK14 were available.

Three of the five whales that started the study as juveniles and were not most closely statistically linked to their mothers were females, AK7, AN8, and AN11, and produced offspring during the study. They were more closely bonded to their offspring, rather than their own mothers and demonstrate the process of new mothers developing distance from their own mothers as they produce calves. Another of these juveniles, AB18, lost its mother early in the study and it became most closely linked with a siblings offspring, AB41. Young whales that lose their mothers may travel with close relatives or may wander between subgroups. Finally, the juvenile male, AN19, was more closely bonded to apparent sibling, AN18 (also a juvenile at the begining of the study), than to his apparent mother AN17, for reasons that are not clear.

Bonds between females and their mothers tended to weaken after the daughter began to produce her own offspring (Table 4). For example in the 1984-88 analysis apparent mother, AN 9, and daughter, AN35, were linked at CAI 29, in the 1889-95 analysis they were linked at CAI 2. AN35 produced viable calves in 1988 and 1991. In a more extreme example, apparent mother AN9 and daughter AN10 were linked at CAI 22 in the 1984-89 analysis and in the 1989-95 analysis they were linked at CAI -20. AN10 had viable calves in 1987 and 1992. The tendency of reproductive females to travel more distantly from their mothers suggests a process basic to new pod formation.

Bonds between males and their mothers varied in strength and appeared somewhat independent of age (Table5, Figure 9). For example AN19, (est. age 22 yr) has a relatively weak bond (CAI 16) to his apparent sibling AN18 (est. age 16 yr) and an even weaker bond (CAI 4) with apparent mother AN17. Another old male in the same pod, AN4 (est. age 33+ yr.), has a very strong bond (CAI 49) with his apparent mother AN27, despite his relatively old age Adult males generally demonstrated a weaker bond with an adult sister than with a mother. This was helpful in constructing genealogical trees where the mother had died. For example, AE 3, a male that matured during the

study was more strongly bonded (CAI 38) to his probable mother, AE 4 (dead), than to his probable sister, AE2 (CAI 16).

Direct observation indicated that some males occasionally traveled with other adult or juvenile males or traveled independently particularly during the multipod encounters observed in July and August (Matkin et *al.*, 1997). Intrapod groups dominated by adult males (ie. AB10 intrapod group) also tended to travel more independently.

As in Bigg et *al.* (1990) the genealogical trees for the intrapod groups were matrilineal groupings of mothers and their descendents. The number of intrapod groups per pod was higher in PWS (range 1-9 mean 4.5) than for British Columbia (range 1-5 mean 2.6) as determined by Bigg et *al.* (1990). However, the number of whales per intrapod group was similar. in Prince William Sound the range was 1-9 individuals (mean 4.2) and in British Columbia the range was 2-9 individuals (mean 3.6). These factors combined reflect a larger average pod size in Prince William Sound.

We were most confident in the genealogical trees for pods that were most frequently photographed, such as AE and AK pods and less confident in the much less frequently observed AD5 and AD16 pods. The large number of mortalities in AB pod also made construction of genealogical trees more difficult for this pod. The greatest potential source of error for genealogical assignments was if the mother of a young whale died prior to the study. In this case the young whale would likely travel with its closest female relative. Since the mortality rate for reproductive females is extremely low (.0048 in Olesiuk *et al.*, 1990), this source of error probably was insignificant.

For the resident pods we examined, the total number of whales increased over the period of the study, indicating that a majority of matrilineal groups were growing and/or dividing over the past decades. However, one pod, ABpod, declined during this period from 35 whales to 23 whales. Six of the mortalities occurred during 1985 and 1986 when there were interactions with the sablefish (*Anaploma fimbria*) fishery (Matkin et al 1994). Apparent bullet wounds were observed on 16 whales during those years. Fourteen of the mortalities occurred in the year and a half following the 1989 *Exxon Valdez* oil spill (Matkin *et al.*, 1994). Some of the matrilineal groups in AB pod are nearly extinct due to these mortalities. An adult male, AB3, is the apparent final member of a once large matrilineal group linked by the apparent sisters AB6 and AB7. Another large matrilineal group (matriarch AB9) has been reduced to a single orphaned 5 year old, AB45. Many of the mortalities have been juveniles (13) or reproductive females (4), severely reducing the reproductive potential of the matrilineal groups.

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AB Pod AB10 Subpod Figure 1.





AB Pod AB17 Subpod Figure 2.

Al Pod



AJPod

Figure 3





Fisure 4



AN20 Pod

Figure S



AK Pod



AE Pod

Figure 6.





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Figure 7.



Determination of Prince William Sound Resident Pods by Association Index (PCC)

Figure 8.

Figure 10





AE14 or



Figure 10

Figure W

AB10 Subpod



B02 B29 B25 B46 B39 B30 B31 B38 B32 B47 B33 B42 100 12 -23 -52 -36 -34 -22 -45 -16 10 -19 -10 B02 100 -10 -44 -47 -28 27 -56 . -65 -74 B29 . 100 65 31 -12 -19 -9 -42 -65 -41 -35 B25 100 17 12 -76 -75 -72 B46 . . 100 6 -29 -10 -51 -68 -37 -43 B39 100 -2 14 2 8 -26 B30 . 100 42 -2 -9 -46 B31 . 100 -54 -72 -53 -39 B38 76 100 37 25 B32 100 25 . B47 AB Pod 1`00 22 B33 AB25 Subpod 100 B42



AB39⁺ (87)

AB46 (92)

AB51 (96)

Flgure II

\$19-11-160



Figure 12





AJ37 (94)

Figure 14

Figure 14

<u>103</u> <u>104</u> 106 105 102 101 100 27 -17 -22 -27 -19 <u>103</u> 100 -14 -35 -37 -29 <u>104</u> 100 -17 -25 -22 106 100 -6 -36 105 100 -2 102 100 101





AN38 (87)

AN46 (92)

Figure 15

100 51 - 100 - 1. Figure 16	-59 -34 -60 -50 -71 -11 00 54 100	-88 -54 -82 95 -64 22 -72 -61 -64 -56 -2 -68 -38 100 41 11 100 12 100	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	-53 -86 N13 -78 83 N15 -40 2 14 N17 -52 52 3 N34 -71 10 5 N21 -89 79 N16 -20 N33 N16 -20 N33 N16 -20 N33 N16 -93 -54 -60 N17 -86 -81 -93 N18 -93 -82 N20 N39 -77 75 N22 -95 -63 -91 N31 - N44 N44 -53 -71 -92 N19 -31 -48 -92 N23
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4 	AN17 ¥	AN33~		• •	AN27 AN23 ⁸	100 7 10 10	00 35 N43 100 21 11 N24 100 54 N30
AN19♂ AN	: 18 A1 *) N37 (87)			AN26 ² AN36 (8 \N42 (90)		100 N28
HGv x 16					• •	AN29 [§] AN24 a ⁿ AN43 (90)	AN28 [°] AN30 <i>°</i> AN34 [°]
				÷.,			

ŧ

AN34⁹ AN21*d*

Figure 14

K02	K13	K10	K09	K05	K04	K03	K06	K11	KO8	K07	K12	K14	KOT	
100	72	54	51	26	29	17	-81	-82	-82	-73	-92		-56	KO2
	100	61	47		21	-34	-93		-92				-87	K13
		100	36		13	12	-94	-87	-85	-83	-92		-76	K10
			100		21	7	-86	-85	-86	-78	88		-71	K09
				100	21	23	-65		-66	-67	-		-56	K05
					100	16	-75	-88	-60	-68			-43	K04
						100	~58	-74	-66	-61	-90		-38	K03
							100	46	34	29	-3		20	K06
								100	18	-12	-10	•	-6	K11
									100	9	2		10	KO8
										100	57	•	11	K07
											100	100	~5	K12
												100		K14
													100	K01
								100 A					-	

AK Pod





Prove of 17 D22 D24 D09 D01 D03 D10 D08 D23 D04 D11 D19 D26 D05 D25 D06 D21 D02 D12 0 60 51 - 1 -4 -52 -35 3 -59 -64 28 -70 -2 -76 -75 -7.4-82 -80 -81 D07 8 -39 100 - 36 12 -87 · . -18 -18 -76 -81 -77 -82 -83 D22 100 13 . . -41 6 -16 -46 -17 -86 . -90 -86 -38 . . -64 D24 100 6 -74 -49 29 -72 4 . -42 -D09 100 2 40 -73 33 -77 . DOT 100 50 -85 -90 44 -89 . D03 100 -71 57 . D10 100 100 6 -17 -28 -20 -77 -91 -78 -75 -46 -63 D08 100 100 -28 -28 -76 -52 D23 100 -20 -30 15 -84 AD5 Pod -67 -51 D04 100 55 35 -78 -68 -73 -32 FIGURE 18 . -48 D11 100 47 -67 -90 -73 -59 -66 -51 D19 100 -91 -83 -57 -74 -54 -29 D26 100 75 68 56 7 -24 D05 AD2 & ---- AD5 \$ 100 30 39 -8 -69 D25 100 34 4 -33 D06 . . : • • • 11 -50 D21 100 100 59 D02 AD12 & AD6 ~ AD21 (90) AD25 (95) 100 D12 6 D20 D18 D13 D17 D14 D15 9 -42 -24 0 22 AD4 -45 -17 D16 AD119 -3 -50 100 -50 D20 . 100 -34 -11 -37 -20 D18 100 37 39 -6 D13 AD3 ° AD10 ADIS 100 12 -23 D17 AD19♂ AD26 (89) AD27 (96) 100 -14 D14 100 D15 AD9 1 1 AD14 Pod 1 AD7 ٩ AD15 ---- AD20

AD8 + AD24 (90) AD22 (93-4)

AD23 (94)

D18

AD20

AD13

AD17

AD14

Appendix 3

Foraging strategies of sympatric killer whale (Orcinus orca) populations in Prince William Sound, Alaska

Marine Mammal Science

Foraging strategies of sympatric killer whale (Orcinus orca) populations in Prince William Sound, Alaska

1

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Abstract

The foraging behavior of sympatric resident and transient killer whale populations in Prince William Sound, Alaska was documented from 1984-1996. Residents spent 35% of their time and transients spent 52% of their time foraging. Transients preyed exclusively on marine mammals while residents preyed exclusively on salmon (Oncorhynchus spp.) and herring (Clupea harengus). Of observed predations, 33% were of harbor seals (Phoca vitulina), 39% were of Dall's porpoises (Phocoenoides dalli), and 6% were of harbor porpoises (Phocoena phocoena). The Gulf of Alaska and the AT1 transient populations preved upon Dall's porpoises, while only AT1 transients preved upon harbor seals. Forty-three harassments of marine mammals by transients were observed, 32.6% of which were of Steller sea lions (Eumetopias jubatus) by Gulf of Alaska transients. Sixty-three salmon scale samples were collected from resident killer whale predations. Ninety-five percent of these were identified as coho salmon (Oncorhynchus kisutch) scales. Resident killer whales interacted with Steller sea lions and Dall's porpoises on 66 occasions; none of these interactions involved predation. In conclusion, residents and transients in Prince William Sound exhibit dietary specializations which explain differences in the social organization and behavior of the two types of killer whale.

Key Words: killer whales, Orcinus orca, Prince William Sound, foraging, predation, behavior

Introduction

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Two sympatric forms of killer whale have been identified in Prince William Sound, Alaska: resident (fish-eating) and transient (mammal-eating) (Ellis 1987; Heise *et al.* 1992). These two 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), Washington State (Balcomb *et al.* 1982), and southeastern Alaska (Matkin and Dahlheim 1995). The two forms have not been seen in association with one another in any area where they have been studied (Ford *et al.* 1994; unpubl. data).

At least three populations, two of the transient form and one of the resident form of killer whale, have been proposed for Prince William Sound, based on genetic analysis (L. Barrett-Lennard, in prep.), social characteristics (Saulitis 1993: Matkin and Saulitis 1994), acoustics (Saulitis 1993: Barrett-Lennard *et al.* 1996; unpubl. data), and morphology (Baird and Stacey 1988). Resident killer whales travel in groups called pods, which exhibit long-term stability (Bigg *et al.* 1990; Matkin *et al.*, in prep.). Resident killer whale pods in Prince William Sound range in size from seven to 36 (Matkin *et al.* 1994; unpubl. data). Prince William Sound resident pods have been seen in association with residents from outside Prince William Sound (Matkin *et al.*, in prep.), but have never been seen in association with transients (unpubl. data).

At least two separate populations of transient killer whales use Prince William Sound. The AT1 group and the Gulf of Alaska transients have never been seen in association with one another and are distinguishable by differences in mitochondrial DNA (L. Barrett-Lennard, in prep.) and acoustical characteristics (Saulitis 1993; unpubl. data). Other transient groups have been seen in Prince William Sound rarely and have been tentatively classified as transients by examination of morphological characteristics. These transients have not been genetically or acoustically sampled and their association patterns are unknown; they have not yet been assigned to populations.

Members of the AT1 group of transients (22 whales in 1984) are seen regularly in Prince William Sound and is seen there year-round (Matkin and Saulitis 1994; unpubl. 3

data). The Gulf of Alaska transients are seen infrequently in Prince William Sound; their range is unknown (Matkin and Saulitis 1994).

Dietary specialization is the key factor explaining the behavioral differences between residents and transients. Resident killer whales in British Columbia and Washington State waters feed exclusively on fishes; transients feed exclusively on mammals (Ford *et al.*, in prep.).

The recognition of two distinct forms of killer whale that exhibit dietary specializations has challenged the view that killer whales are opportunistic predators (i.e., Rice 1968). Nonetheless, killer whales have been reported to feed on nearly every marine mammal species available to them (Hoyt 1984; Jefferson *et al.* 1991; Matkin and Saulitis 1994), and in the North Pacific, they have been reported to feed on seventeen species of fishes (Pacific Biological Station, Nanaimo, B.C., unpubl. data).

The feeding ecology of sympatric resident and transient killer whale populations has been described only for killer whales observed between southeastern Alaska and Washington State (Ford *et al.*, in prep.). In this paper we present fourteen years of data on the dietary and behavioral differences between resident and transient killer whales in Prince William Sound, Alaska. Dietary differences between residents and transients in Prince William Sound are compared to those described er ewhere. We examine possible specialization in prey choice by marine mammal-eating killer whales. Finally, the differences in social organization and behavior of resident and transient killer whales in Prince William Sound are discussed in light of their unique dietary specializations.

Materials and Methods

Data were collected over an area of approximately 3500 square km, in Prince William Sound, Alaska, although most of the effort was concentrated in the southwestern part of

Prince William Sound, including Knight Island Passage and Montague Strait and bounded by the Gulf of Alaska (Figure 1).

Feeding behavior was documented during annual photo-censusing for population monitoring from 1984-1996. All observations were made at sea from several boats ranging in size from 4.7-12.8 m from late March through October.

Although months spent in the field varied among years, data collection occurred during July and August in all years of the study. Killer whales were located by visual searches. acoustic detection, and by responding to VHF radio reports from other vessels.

The natural markings on the dorsal fin and saddle patch areas are unique to each killer whale (Bigg *et al.* 1987). During each encounter with whales, we attempted to photograph the left-hand dorsal fin and saddle patch of every killer whale present using the method of photo-identification described by Bigg *et al.* (1986). Identified individuals were categorized into pods (residents) and groups (transients) based on repeated associations among whales(Ellis 1987; Heise *et al.*, 1992).

Data were recorded on standardized forms and included the date, beginning and end location and time of encounters, a summary of acoustical recordings made and of identification photographs taken, and field identifications of whales present. During the encounter, the whales' travel route during was drawn of a map attached to the encounter form.

Behavioral observations were made on a continuous basis, by scanning the activities of the entire group (scan sampling: Altmann 1974). The beginning and end times of activity states and descriptions of the whales' specific behaviors, including evidence of predation, were recorded. The behavior of the whales was categorized into standardized activity states used in mammalian behavioral studies (foraging, resting, traveling, socializing) (Dunbar 1988). Most other killer whale studies have used these four general activity states as well (Ford 1989; Morton 1990; Felleman *et al.* 1991; Barrett-Lennard 1996). These categories are defined as follows:



Figure 1. The study area in southwestern Prince William Sound, Alaska.

Socializing - Whales interacted with one another physically. This included sexual behavior and a variety of other behaviors such as chasing and rolling. Aerial displays not related to feeding were commonly observed. These included breaching, spy-hopping, and fluke- and flipper-slapping.

Foraging - This category included the search for, pursuit of, capture, and consumption of prey.

Traveling - Traveling whales moved in a line-abreast pattern in one or more groups. Members of the groups surfaced and dove synchronously and moved on a consistent compass course.

Resting - The movements and breathing patterns of resting whales were closely synchronized. The whales moved at speeds much slower than those of traveling whales. Resting whales were commonly grouped in maternal units (Matkin *et al.* in prep.) Individuals typically surfaced within a single body length of their neighbors.

When successful predation on salmon was suspected, the kill site was approached slowly. An observer on the bow of the research vessel scanned the area and retrieved salmon scales using a long handled dip-net. The scales 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 by species at the Pacific Biological Station, Nanaimo, B.C. On some occasions, fish kills were confirmed by the observation of fish in the mouths of the whales.

The presence of potential prey in the vicinity of killer whales was noted and the number, species, and behavior of potential prey animals was described. Feeding on marine mammals was indicated by milling and surface activities such as breaches, tail-

slaps, and high leaps. 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. When marine mammal kills were suspected, the kill site was approached slowly. If possible, samples of prey remains were collected and frozen for later identification. Potential marine mammal prey species were considered harassed when they exhibited an avoidance response or alarm in the presence of nearby killer whales.

Behavioral data were entered into a GIS database (Matkin *et al.* 1996) using Arc/Info software. Separate activity budgets were developed for residents and transients. Only behavioral data collected from 1988-1996, when most of the behavioral data were collected by a single observer, were used in statistical tests. Behavioral data before 1988 were collected by numerous observers, and behavioral categories had not been consistently defined. Activity budgets of residents and transients were compared using ANOVAs run on each activity state. P-values of less than 0.05 were considered to be significant and P-values between 0.05 and 0.10 were considered to be marginally significant.

Results

The data presented here represent 662 encounters with killer whales from 1984-1996. Transient killer whales were encountered on 196 occasions: 466 encounters were with residents. Behavioral data were collected during 2429 hours of observation, 515 with transients and 1914 with residents.

The AT1 group was the most commonly seen transient group (n= 174 encounters). Gulf of Alaska transient groups (AC,AU,AT60,AT80) and unclassified transients 8

(AT30,AT50,AT70) were seen rarely in Prince William Sound during the study (n = 22 encounters).

Residents spent significantly more time resting than transients (p = 002; Figure 2). Residents spent more time socializing than transients (p = 0.082) and transients spent more time foraging than residents (p = 0.078). 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). The activity budgets of Prince William Sound resident and transient killer whales differed from those developed from studies off British Columbia and Washington State (Table 1).

Killer whales used three foraging strategies in this study: offshore foraging, nearshore foraging, and foraging for fishes. Offshore foraging was observed only in transient killer whales hunting marine 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 a km or more beneath the surface at times, often during dives of ten-minute or longer duration. When prey were detected, a coordinated chase involving all whales in the group ensued, and prey were shared among group members. Offshore foraging involved an average group size of 5.4 whales/group, and attacks on Dall's or harbor porpoises occurred frequently during this type of activity. The whales were generally silent during offshore foraging (Saulitis 1993). Passive listening may be employed in the detection of prey during both nearshore and offshore foraging (Saulitis 1993; Barrett-Lennard 1996).

Nearshore foraging was observed only in transient killer whales hunting marine mammals, predominantly harbor seals. Whales closely followed the contours of the coastline, remaining within 20 m of shore. They often entered small bays and narrow channels and explored rock outcrops and shoal areas. The whales were generally silent (Saulitis 1993). Nearshore foraging involved an average group size of 3.3 whales/group



Figure 2. Activity budgets of resident and transient killer whales in Prince William Sound, Alaska. 1988-1996.

Table 1. Comparative percentages of time spent in four activity states by North Pacific resident and transient killer whales.

LOCATION								
	Vanc. I ¹	Vanc. I ²	Vanc. I ³	Pug. Sd.	PWS			
BEHAVIOR			. ⁶ . 1					
Travel	4.2	8.0	-	25.0	35.2			
Rest	13.2	21.0	-	13.0	17.6			
Forage	66.5	50.0	-	47.0	35.5			
Social	11.6	21.0	-	15.0	11.7			
N (hours)	416	243		985	1914			
		TRAN	ISIENT					
Travel	15.0	12.0	31.4		38.5			
Rest	0.0	6.0	1.7		4.1			
Forage	77.0	81.0	63.1	~	50.0			
Social	8.0	0.0	3.8	-	7.4			
N (hours)	101	43	434	-	515			

¹Vanc. I. = Vancouver Island: Ford (1989): ²Vanc. I.: Morton (1990): Pug. Sd. = Puget Sound: Felleman *et al.* (1991); ³Vanc. I.: Baird (1994): PWS = Prince William Sound. Alaska: this study. and these groups often split into still smaller groups, with individuals exploring different parts of the shoreline.

Foraging for salmon was identified by characteristic surface behaviors, including tight circling, rapid and erratic movements, and lunges. Chasing and capture of salmon was accomplished individually, by mother-offspring groups, or, rarely, by pairs of juveniles. Other cooperative foraging for salmon was not observed. Echolocation clicks were emitted during foraging for salmon. During this type of foraging, killer whale groups generally dispersed widely, sometimes over several square kilometers.

We never observed predation or attempted predation on marine mammals by resident killer whales. Thirty-one kills of marine mammals by transient killer whales were accumented. Transients preyed almost exclusively upon Dall's porpoises and harbor seals (70.9% of kills; Figure 3). 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 described as unidentified marine mammals (n = 2) or unidentified pinnipeds (n = 1).

Most harbor seal kills (n = 11 total) occurred beneath the water's surface. In contrast, Dall's porpoises kills involved highly visible surface obc All but three harbor seal kills occurred during nearshore foraging and all Daul se 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.

Harbor seals reacted to the presence of transients by swimming toward or climbing on shore, by remaining still in shallow water, and by hiding around the research vessel. Steller sea lions reacted to the presence of transients by barking loudly and becoming agitated on haul-outs, climbing onto or hiding near shore, switnming away from the whales rapidly, forming tight groups in the water, and craning their necks to watch the movements of the whales, at times charging towards them or charging away. Dall's 12


Total Transient Kills

Figure 3. Diet of transient killer whales in Prince William Sound, Alaska based on thirtyone documented kills, April-October, 1984-1996. Numbers of kills observed were: Dall's porpoise, n = 12; harbor seal, n = 10; harbor porpoise, n = 2; unidentified marine mammal, n = 7.

porpoises reacted to approaches by transients by fleeing rapidly ("porpoising") or by swimming very quietly at the surface when transient killer whales were nearby.

Forty-four harassments of marine mammals by transient killer whales were documented (Figure 4). Most harassments were of Steller sea lions (n = 14) and harbor seals (n = 12). 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.

The AT60 group, which is part of the Gulf of Alaska transient population, was seen on 14 occasions: in all but three of these, they were observed in the vicinity of the Steller sea lion haul-out at the Needle, in Montague Strait, in southwestern Prince William Sound. Although successful attacks were not observed, during all of these observations. Steller sea lions appeared agitated by the presence of the whales.

Transient killer whales were never observed preying on fish; however, in one instance, an AT1 individuals chased a salmon beneath the research vessel.

Scale samples were collected from fish kills made by 63 resident killer whale in five years of the study (1991-2; 1994-6). Ninety-five percent of the scale samples were from coho salmon (Table 2). The rest of the scale samples were from chinook (O. *tshawytscha*) and chum (O. *keta*) salmon. Nineteen scale samples were collected from unidentified resident whales. Most samples (n = 29) were collected in August.

On 37 occasions, predation on fish by resident killer whales was observed but scale samples were not collected. These predations were confirmed by the observation of fish in the mouths of whales, or by observation of fish parts in the water near the sites of suspected kills. Thirty-six were on salmon and one predation was on herring. Observations were made in 1984, and 1989-1996, from May through September.

Resident killer whales interacted 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 (*Megaptera novaeangliae*), a minke



Figure 4. Harassments of marine mammals and fishes by transient killer whales from the AT1 and Gulf of Alaska (GOA) populations. from 44 observations, April-October, 1984-1996, Prince William Sound, Alaska.

Table 2. 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 from28 different identified resident killer whales representing seven pods.

Pod	Number of Samples	Species
AB	12	coho
AN	3	2 coho, 1 chinook
AI	3	coho
AE	20	coho
AJ	3	coho
AK	5	3 coho, 1 chum
AD	2	l chinook

whale (*Balaenoptera acutorostrata*), and a sea otter (*Enhydra lutris*) 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 Dall's porpoise remained with the AB pod of resident killer whales for the course of an entire summer. Steller sea lions interacted with resident killer whales by surfacing among them, porpoising towards them, nipping at them, or by surfacing alongside individual whales. Dall's porpoises and Steller sea lions were seen among all of the resident killer whale pods we have identified in Prince William Sound, during the April through September period. Interactions occurred during all four general killer whale activity states.

Discussion

1

The activity budgets of Prince William Sound resident and transient killer whales differed from those developed from studies off British Columbia and Washington State (Ford 1989; Morton 1990; Baird 1994). Transient killer whales in Prince William Sound spent less time foraging and more time traveling than did transients off Vancouver Island and in Puget Sound. However, traveling whales may be alert to predation opportunities, and therefore some traveling behavior may also function as foraging (Saulitis 1993: Barrett-Lennard 1996). The higher percentage of time spent traveling by transients in Prince William Sound may also be indicative of more widely dispersed food resources in this area.

In southwestern Prince William Sound, harbor seals were scattered through out the study area on many small haul-outs, three of which had over 50 seals/haul-out: the

northwest corner of Montague Island, northern Prince of Wales Passage, and the fjord system of Icy Bay (Figure 1).

In the Gulf of Alaska/Aleutian Islands, trend counts during the molting period for harbor seals declined by 19% from 1989-1995; counts made during the pupping season declined by 31% (Hill *et al.* 1996). The Gulf of Alaska/Aleutian Islands harbor seal population is estimated at 23,500 (Hill *et al.* 1996). Harbor seals around Kodiak Island have declined an estimated 85% between 1976 and 1988 (Pitcher 1990).

The most recent population estimate of harbor seals in Prince William Sound is 5,300 (Frost *et al.* 1996). Most of the population is concentrated on the eastern side of Prince William Sound. While recent counts suggest a leveling off of the decline in the Gulf of Alaska (B. Kelly, pers. comm.), 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 Knight Island area in the last decade (M. Eleshansky, pers. comm.). The widely dispersed nature of the harbor seal population in Prince William Sound and its state of decline may increase the amount of time transients spend traveling through the study area, moving between areas of prey abundance.

Harbor seals in British Columbia waters have been increasing exponentially (12.5% per year) since they received protection in 1970 (Olesiuk *et al.* 1990). The population of harbor seals off the British Columbia coast was estimated to be between 75.000 and 88,000 in 1988 (Olesiuk *et al.* 1990).

Transient killer whales in that area spend over 60% of their time foraging and over 95% of the kills identified by Baird (1994) were of harbor seals.

Transient killer whales in Prince William Sound preyed primarily on Dall's porpoises and harbor seals. While transients employ stealth and passive listening in hunting both species (Saulitis 1993; Barrett-Lennard 1996), attacks and kills are accompanied by increased vocal and surface activity (Saulitis 1993). Once a Dall's porpoise kill has been made, transients may have to travel some distance to encounter porpoises that have not been alerted to their presence. Dall's porpoises made up a larger proportion of the diet of transients in Prince William Sound (39%) than in British Columbia (6%) and a consequence may be increased travel time for Prince William Sound transients.

Travel time has been found to be related to prey distribution in other mammalian species. Two species of hyena inhabiting the same region of the Kalahari Desert in southern Africa spend different amounts of time traveling (Mills 1989). The territory size of the spotted hyena (*Crocuta crocuta*), which feeds on large and medium-sized ungulates, is much larger than that of the brown hyena (*Hyaena brunnea*), which feeds on small food items such as fruit, insects, and small mammals. The average distance traveled between meals is greater for spotted than for brown hyenas. Considerable intraspecific variation in behavioral and morphological characteristics has been found in many carnivore species (Beckoff 1989).

Resident killer whales in Prince William Sound appear to spend more time traveling and less time foraging than residents off British Columbia and Washington State. These differences may be due, however, to the difficulty in clearly distinguishing between foraging and traveling. Resident killer whale group size — re significantly larger than those of transients (Morton 1990). Resident killer was — ds are often widely dispersed over several square kilometers during foraging and social activity, and several behaviors may occur simultaneously. While differences in the activity budgets of resident whales from different areas are likely to exist, quantitative comparisons are not possible.

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 in this study were similar except that transients and residents spent an equal amount of time traveling.

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 (Jacobsen 1986, 1990; Osborne 1986). The functional significance of this behavior is unknown, but it may help to reinforce the strong social bonds within resident pods (Jacobsen 1990). Resident pods that rest together show a high degree of association throughout the season. Transient killer whales have a more fluid group membership, and group resting may not have the same social significance.

In all studies of killer whale behavior in the North Pacific, including this one, transients spent less time socializing than did residents (Ford 1989; Morton 1990; Baird 1994). In Prince William Sound, time spent socializing ranged from 0% to 12.6%, except in 1988 (32.1%). Socializing transients are generally vocal and engage in highly visible surface behaviors (Saulitis 1993), while small groups of foraging transients are much more difficult to spot by the novice observer. In the first years of the study, our detection and observation of transient killer whale may have been biased toward the more conspicuous social behaviors.

Several factors may be responsible for less socializing by transient killer whales. Social behaviors, with their attendant surface and vocal activity, may increase the chances that potential marine mammal prey, which have acute abilities, could be alerted to the presence of transient killer whales. Additiona finding and capture may be more energetically costly for transients than for residents. During the summer months, mixed schools of salmon enter Prince William Sound in large numbers by predictable routes. For residents, the abundance and predictable occurrence of a food supply may increase the energy available for activities such as social play and sexual activity (Kano 1992). Transients may devote so much time and energy to food-finding, that they do not have surplus energy for social activity.

The time spent in social activity in primates decreases with the amount of time spent moving and feeding (Dunbar 1988). The cost of play is unclear (Bekoff and Byers 1988).

but this activity is often curtailed when food supplies decrease (Martin 1982; Harcourt 1991).

Play in vervet monkeys (*Cercopithecus aethiops*) is influenced by time budgets, energy available in the diet, and the overall abundance of their food resources (Lee 1984). The time spent socializing by killer whales may likewise be related to the cost of this behavior in relation to the amount of time required to find food. Nearly all social activity by transients occurs following kills (Morton 1990; this study).

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; Barrett-Lennard *et al.* 1995). Observations of feeding killer whales in Prince William Sound support this assertion, where fish-eating and mammal-eating forms occur sympatrically, but do not associate.

The stomach contents of five killer whale carcasses recovered in or near Prince William Sound reflect a pattern of feeding segregation (Heise *et al.*, in prep.) Three stomachs contained marine mammal remains, one contained two circle hooks used in the commercial long-line fishery, and one stomach was empty (Heise *et al.*, in prep.). The three stomachs with marine mammal parts contained remains of Steller sea lions (two stomachs), harbor seals (three stomachs), Dall's porpoise (one stomach), and harbor porpoise (one stomach) (Heise *et al.*, in prep.).

Ford *et al.* (1995) summarized observations of predation events and the 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-nine percent of the observed prey were harbor seals and 5.7% were Dall's porpoises (J. Ford, pers. comm.). Transients in those areas also killed and consumed seabirds. Transients were never observed to kill fish; no transient stomachs contained fish remains. 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 1990, 1991; Guinet 1990, 1991). Off Crozet, intentional stranding behavior is performed by adult females preying upon southern elephant seals (*Mirounga leonina*) (Guinet 1991). 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 1990, 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 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.

Observations by reliable observers indicate that juvenile Steller sea lions become more abundant in Prince William Sound with the arrival of herring in the 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 (Heise *et al.*, in prep.). Observations of harassment of Steller sea lions by the AT1 population suggest that they may occasionally prey upon Steller sea lions as well. Barrett-Lennard *et al.* (1995) estimated that Steller sea lions make up 25% of the diet of killer whales in Alaska. Steller sea lions made up 6.6% of the diet of transient killer whales off British Columbia, Washington State, and southeastern Alaska (J. Ford, pers. comm.).

Some killer whale groups 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 the technique of intentional stranding, a highly risky behavior that sometimes results in killer whale mortality, from their mothers (Guinet 1990). 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. Specific groups of killer whales in British Columbia specialize on a particular type of harbor seal foraging, termed "haul-out foraging" (Baird 1994). In our study, the AT60 group was consistently observed harassing Steller sea lions around the Needle, a haul-out in southwestern Prince William Sound. No other transient killer whale group was observed foraging around the Needle or other Steller sea lion haul-outs.

Steller sea lion predation may involve considerable risks to killer whales due to the large size and aggressive nature of adult sea lions. Steller sea lions were observed charging toward both resident and transient killer whales arransient killer whales also were aggressively approached by adult humpback when the potentially dangerous prey species.

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. Our predation data is biased in its seasonality; observations were made from April through October, with most observations made from May through September. The winter diet and foraging behavior of killer whales using Prince William Sound has not been documented.

There have been reports of killer whales attacking humpback whales in Prince William Sound (N. Naslund, P. Kompkoff, pers. comm.). The observations of

harassments of humpback whales by transient killer whales and the documentation of killer whale teeth marks on the flukes of humpback whales in Prince William Sound (von Ziegesar 1991) indicate that this species may be a component of the transient killer whale diet. Killer whale predation on gray whales (*Eschrichtius robustus*) is frequently reported in western Alaska (Fay *et al.* 1979; Lowry *et al.* 1987; B. Laukitis pers. comm.). Killer whales have been reported to feed upon all cetacean species available to them in the North Pacific (Matkin and Saulitis 1994).

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 13.1% of the diet of transient killer whales off British Columbia. Washington State. and southeastern Alaska (Ford, pers. comm.).

The extensive catalogue of documented prey (Hoyt 1984: Jefferson *et al.* 1991; Matkin and Saulitis 1994) suggests that killer whales likely exhibit some degree of behavioral flexibility, as evidenced by the AT1 population use of two very different foraging strategies to hunt harbor seals and Dall's provide 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 and Aleutian Islands and Prince William Sound may result in killer whales using different strategies to exploit alternative species.

Though resident killer whales off the coasts of British Columbia, southeastern Alaska, and Washington State prey upon all five species of Pacific salmon, they appear to prey preferentially on chinook salmon (Ford *et al.* 1995). Chinook salmon scales make up 64% of the samples collected in those areas, and stomach content analyses also reflect a preference for chinook salmon (J. Ford, pers. comm.).

Chinook salmon are rare in southwestern Prince William Sound during July and August. 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. 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.

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. However, our scale sample data reflect only those kills that were made at the surface, which may create a bias for the observation of coho salmon predation and against the observation of predation on other salmon species.

While there is little of no 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, and are not found in waters cooler than 7 C (XX Pac Salm Life Hist).

Chum and sockeye salmon prefer the coldest water temperatures of the five Pacific salmon species. and chinook salmon are found at the greatest depths (Pac Salm Life Hist). Off northern Japan, chum salmon descend to waters below 100 m. apparently in response to temperature (Veno 1992). During May and June, when chum and sockeye salmon return to Prince William Sound, we observed killer whales foraging for salmon without surface chasing of fish. During these encounters, we were unable to obtain scale samples, but suspected that chum or sockeye salmon were the prey.

Chum and chinook salmon may dive to depth to avoid predation by killer whales; they dive deeper than other salmon species when encountering commercial salmon seines, while coho salmon tend to remain at the surface, working their way around nets (G. Ellis, C. Matkin, pers. obs.).

Sockeye salmon contain the second highest amount of fat of the five Pacific salmon species (Sidwell 1981; Exler 1987). While they are smaller in size than coho salmon, they contain a comparable amount of fat per fish; however. no sockeye predation was documented in this study. Off British Columbia, southeastern Alaska, and Washington State, sockeye salmon make up only 3.9% of documented predations (J. Ford, pers. comm.). It is possible that resident killer whales in Prince William Sound feed on sockeye salmon at greater depth, making scale collection difficult. The escape response of sockeye salmon may also make them more energetically costly to pursue. Observations by some of the authors suggest that sockeye salmon are faster than cohos and travel in large schools that may contribute strongly to a confusion effect in response to predators (C. Matkin, G. Ellis, pers. obs.).

Pink salmon are the smallest and lowest in fat content of the five Pacific salmon species (Exler 1987: Sidwell 1981). They comprised 15% of scale samples collected at sites of killer whale predation off British Columbia. Washington State, and southeastern Alaska (J. Ford. pers. comm.). 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.

Some 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 (similar in size to chum salmon) (S. Morestad, pers. comm.) of the five salmon species found in Prince William Sound, and contain the third highest amounts of protein, fat, and calories (Pac Salm Life Hist; Sidwell 1981; Exler 1987).

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 (1996) describes 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 which 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 1996), while small group sizes may enhance the hunting success of transients, which depend upon stealth to capture marine mammal prey (Baird 1994).

Clearly, killer whales in Prince William Sound exhibited distinct dietary specializations similar to those described for killer whales off British Columbia, Washington State, and southeastern Alaska. Each of the cree populations of killer whales in Prince William Sound identified through control andrial DNA analysis (Barrett-Lennard, in prep.) exhibited different dietary preferences. The AT1 transients preyed primarily on harbor seals and Dall's porpoises. The Gulf of Alaska transients preyed primarily on Steller sea lions and Dall's porpoises, and the resident population fed primarily on coho salmon. Dietary preferences of residents and transients in Prince William Sound differed from those proposed for killer whales off British Columbia and Washington State. There, resident killer whales prey primarily on chinook salmon, while the transient population, which is genetically distinct from AT1 and Gulf of Alaska transient populations, feeds primarily on harbor seals (Baird 1994; Ford *et al.* in prep). 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.

Appendix 4

Genetic isolation between sympatric ecotypes of killer whales in Prince William Sound, Alaska

Molecular Ecology

Genetic isolation between sympatric ecotypes of killer whales in Prince William Sound, Alaska

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Introduction

Long term studies have shown that two strikingly different assemblages of killer whales inhabit the waters of Prince William Sound. The members of the two assemblages differ in feeding habits, call repertoires, and diet (Matkin, 1994, Saulitis 1993). Social mechanisms effectively prevent association between them, and members of different assemblages have never been seen mixing non-aggressively. A parallel dichotomy exists in the nearshore waters of British Columbia, where two sympatric assemblages are referred to as "resident" and "transient" (summarised in Ford et al. 1994). Based on their similarities to the coastal British Columbian groupings, killer whales in Prince William Sound are also generally referred to as resident and transient.

This study was initiated in 1994 for several reasons. Firstly, reliable knowledge of killer whale population structure would make it possible to better understand the role of killer whales in the ecosystem. This information is of particular interest now, since only transient-type killer whales prey on locally- depleted harbour seals. Secondly, knowing the discreteness of the populations, along with their numbers, demography, and life history parameters would help us assess their sensitivity to disasters such as the Exxon Valdez oil spill of 1989. Thirdly, characterizing Prince William Sound whales genetically would also make it possible to link them to whales from other regions, to determine whether they should be considered for management purposes as local stocks, or as representatives of larger stocks. Finally, the analysis would allow us to determine whether residents and transients comprise two long-standing lineages that populated the coastal waters of the north west Pacific independently, or whether a generalized form bifurcated into specialist forms more than once.

The genetic analysis described here focused on mitochondrial DNA (mtDNA). 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, and is usually the first, and sometimes the only, marker used in population studies. Additional analysis of nuclear DNA is required when patterns of paternally-mediated gene flow or close relationships within populations are of interest.

We initially planned to use restriction fragment length polymorphism (RFLP) analysis for this part of the study. In this method, mtDNA is cut at specific sequences with restriction enzymes. The resulting fragments are sized on an electrophoretic gel, and provide an indirect way of identifying DNA sequence differences. It soon became apparent, however, that this approach would not produce the resolution the study required, because mtDNA diversity in the sampled populations was low. We therefore opted to improve the resolution of the analysis by directly sequencing the mtDNA Dloop region.

Materials and Methods

Biopsy Samples

Researchers aboard small vessels used lightweight biopsy darts to acquire skin samples, following the method of Barrett-Lennard et al. (1996). The system has proven to be efficient and to invoke minimal responses in killer whales in British Columbia (Barrett-Lennard et al., 1996). We biopsied recognized individuals only, photographing them when possible to confirm identities. Skin samples were stored at 4° C in a solution of dimethylsulphoxide and sodium chloride (Amos and Hoelzel 1991), and shipped to the University of British Columbia for genetic analysis. DNA was obtained from the samples by protein digestion, phenol-chloroform extraction, and alcohol precipitation following standard protocols.

Genetic Analysis

To conduct the mtDNA sequencing, we (1) amplified the entire mtDNA D-loop region

with the polymerase chain reaction (PCR), using custom-designed primers based on published mtDNA sequences of other cetacean species (eg Arnason et al 1991); (2) purified the amplified product using QIAQuick® spin columns supplied by Qiagen, Ltd; (3) ran sequencing reactions using Fs-Taq® system reagents and protocols supplied by Applied Biosystems, Ltd, and (4) resolved the sequences using an Applied Biosystems 377 automated DNA sequencer. Because the amplified DNA fragment was too long (943 base pairs) to be entirely resolved in one direction, we obtained the complete sequence by running two reactions, one from each end of the fragment. The sequences were checked by eye and then aligned using the program CLUSTRAL W. Sites found to differ between the sequences were rechecked by eye.

We used a maximum likelihood inference method (reviewed in Swofford et al. 1996) to develop and evaluate hypotheses concerning historical relationships between killer whale groups. The sequences from this study were analyzed along with sequences from British Columbian and north Atlantic killer whales using the phylogeny inference software package PHYLIP (Felsentstein 1993). The procedure used was as follows: the sequences were bootstrapped (randomly resampled with replacement) 100 times, a maximum likelihood algorithm was used to calculate an unrooted tree for each set of bootstrapped sequences, and a consensus tree was calculated based on the 100 maximum likelihood trees.

Results

Biopsy samples

From 1994 to 1996 we acquired genetic samples from 54 Prince William Sound killer whales. All of the samples were unambiguously assigned to photo-identified individuals with the exception of two that were known to have come from one of several identified animals in the same pod. DNA was also obtained from tissue from six killer whale carcasses found in Prince William Sound and adjacent waters. Of the total of 60 DNA samples, 44 were from residents, six from the AT1 group (commonly found in

Prince William Sound), five from the Gulf of Alaska transient assemblage, and five from unidentified whales (all carcasses). An average of 100 μ g of DNA per sample was obtained from the skin biopsies, and approximately 15 μ g of partially-degraded DNA was obtained from each of the carcass samples.

Genetic Analysis

Because mtDNA is inherited maternally, mothers and offspring normally have identical mtDNA sequences. We therefore selected a single individual from each set of maternally-related individuals for sequencing. We sequenced all biopsied individuals for which maternal relationships were not known. The 40 whales sequenced are listed in Table 1.

Group type	Pod§	Individuals sequenced		
AT1 transients		AT1, AT9, AT10, AT14, AT18, AT19 (carcass		
Gulf of Alaska transients		AT64, AC2, AU2, AU3, AU4		
Residents	AB AD AE AG AI AJ AK AN AS	AB3, AB4, AB5, AB14, AB17, AB26 AD4, AD11 AE1, AE5, AE10, AE19, AE20 AG3 AI2, AI3 AJ16, AJ17 AK1, AK8 AN1 AS12, AS-female*, AS-male*		
Unknown		5 samples from carcasses		

 Table 1.
 Killer whales analyzed for mtDNA D-loop sequences

transients into pods.

* Known to be from AS pod but not individually identified.

When we aligned the mtDNA sequences of Prince William Sound killer whales, we

found eight variable nucleotide sites, comprising one insertion/deletion and seven purine-purine or pyrimidine-pyrimidine transitions. The differences at these sites separated the sequences into four haplotypes. One of the haplotypes was common to all members of the Gulf of Alaska group, the second to all members of the AT1 transient group, the third to all members of 6 resident pods, and the fourth to all members of four resident pods. No statistical treatment of these data are necessary to show differences in haplotype frequencies, since no haplotypes are shared between the four groupings. In British Columbia four haplotypes have also been found, two of which are the same as two of the Prince William Sound haplotypes. A haplotype identified in north Atlantic killer whales was not present in the British Columbian or Alaskan killer whales (L Barrett-Lennard, unpublished data). When all seven haplotypes were compared, the number of variable sites increased by 3 (two transitions and a purine-pyrimidine transition). These results are summarised in Table 2, and the maximum likelihood tree based on the consensus of 100 bootstraps is presented in figure 1.

Group	Range	Haplotype [§]	Pods with the haplotype	Number Sequenced‡
British Columbia Northern Residents	central Vancouver I. to central part of Alaskan panhandle	NR	all 15 pods sequenced	25
Prince William Snd. Residents (1)	Prince William Snd. and adjacent waters	NR	AB, AG, AI, AJ, AK. AN	12
Prince William Snd. Residents (2)	Prince William Snd. and adjacent waters	SR	AD, AE, AK, AS	12
British Columbia Southern Residents	Juan de Fuca Str. Georgia Str., Puget Snd.	SR	J (only pod sequenced)	4
Offshores*	pelagic waters from south east Alaska to California	OFF	÷	6
British Columbia Transients	east of 142° longitude to California	BCT	÷	14
AT1 Transients	Prince William Snd. and adjacent waters	ATI	÷	6
Gulf of Alaska Transients	Gulf of Alaska west of 142° longitude	GAT	÷	5
North Atlantic Killer Whales	unknown (sampled whales from Iceland)	ATL	· · · · · · · · · · · · · · · · · ·	2

Table 2Distribution of mtDNA D-loop haplotypes

Pod names from Heise et al. 1991 and Ford et al. 1995.

8 Haplotype designations are based on the initials of the population in which the haplotype was first identified.

* Sequences from British Columbian killer whale populations from an unpublished concurrent study by L. Barrett-Lennard.

* "Offshores" refers to an assemblage of whales found in pelagic waters from British Columbian waters and believed to be socially isolated from members of both the resident and transient groups (Ford et al. 1994).

⁺ Whales in these groups not separated into pods. Each of these groups was monomorphic for a single haplotype.



Gulf of Alaska transients

Figure 1. Consensus of 100 bootstrapped maximum likelihood trees. The numbers indicate the number of bootstraps which had the same combinations of populations to the left and right as shown in the consensus. For example, the Gulf of Alaska transient and the British Columbia transient groups shared an ancestor more recently with each other than with any other group in 78 out of 100 hypothetical trees, and those groups along with the AT1 transients shared an ancestor more recently with each other than with an ancestor more recently with each other than shared an ancestor more recently with each other than with any other group in 99 of 100 trees.

The unidentified killer whale carcasses each had mtDNA D-loop haplotypes matching one of the four haplotypes described above. These matches and the inferred group membership of the dead whales are shown in Table 3.

Location of carcass	Year found	Haplotype ⁺	Inferred group of origin
Culross Island,	1990 Prin	AT1 ace William Snd.	AT1 transient
McLeod Hbr., Prince William Snd.	1991	NR	Prince William Sound residents, Group 1
near Homer, Alaska	early 1990's	GAT	Gulf of Alaska transient
Stockdale Hbr.,	1992 Prin	GAT ce William Snd.	Gulf of Alaska transient
Green Isl., Prince William Snd.	1996	NR	Prince William Sound residents, Group 1

 Table 3.
 Location and year found, mtDNA D-loop haplotype, and inferred group of origin for five unidentified killer whale carcasses.

⁺Haplotype designations as in Table 2.

Discussion

One of the most striking results to emerge from this study is that each group of whales sampled was monomorphic for a single mtDNA D-loop haplotype. This is strong evidence that permanent female movements between the groups is at most extremely rare. The genetically-distinguishable groups map closely onto groups previously-identified based on association patterns and/or acoustic behaviour, as discussed below. While the present study is focused on Prince William Sound killer whales, their inter-relationships are more clear when put into a coast-wide perspective. Thus, we start by describing each of the identified killer whale groups in the north western Pacific.

B.C. Transients

The distinction between transient and resident killer whales was first made by M.A. Bigg and colleagues in the late 1970's (Bigg et al. 1987). These researchers initially

identified two sympatric forms of killer whales based on group sizes, behaviour patterns, and subtle differences in appearance. One of the groups, referred to as *transients*, typically travelled in small groups, and preyed on marine mammals (Morton et al. 1990). Members of the transient group have been sighted from Glacier Bay, Alaska to central California (eg Goley and Straley 1994). We refer to this group as *BC* transients here, to distinguish it from the next two groups. Stevens et al (1989) used RFLP analysis to compare two transient individuals to five sympatric non-transients, and found the first evidence of mtDNA sequence differences between the groups. This result was supported by Hoelzel (1991) who sequenced D-loop region mtDNA from a single transient and two residents, and by L. Barrett-Lennard (unpublished data) with the larger sample sizes listed above.

Gulf of Alaska Transients

Killer whales resembling BC transients in appearance and behaviour have been identified along the coast of Alaska from Prince William Sound to Kodiak Island and further west. It was initially assumed that this group was part of the same transient assemblage seen off British Columbia. However, it was recently determined that no individuals from this group have been identified east of 142° W longitude and that no BC transients have been identified west of the same line (Barrett-Lennard et al. 1995). The maximum likelihood analysis in this study suggests that the Gulf of Alaska assemblage is distinct from the BC transients. The two groups are nonetheless closely related, and we use this as the basis for referring to the Gulf of Alaska assemblage as a transient group.

AT1 Transients

In the mid 1980's a group of 22 whales that were commonly seen in Prince William Sound were identified as being similar in appearance, diet, and behaviour to the BC transients (Leatherwood et al 1984). This group, initially referred to as the AT1 pod, was investigated by Saulitis (1993), who found that it had a unique vocal repertoire, and appeared to be socially isolated from other killer whales. The mtDNA analysis in this study is strong evidence that the AT1's are closely related to both the Gulf of Alaska

transients and the BC transients; accordingly, we argue that is appropriate to refer to them as transients. The genetic and non-genetic evidence of the AT1's unique identity is of particular significance, as the group has recently declined to approximately 10 individuals and has not calved successfully in over ten years.

<u>Offshores</u>

This group of killer whales was not identified until the mid 1980's. It is rarely seen in nearshore waters, and is poorly studied. Its has been sighted in offshore waters from south eastern Alaska to central California (G.M. Ellis, unpublished data). Preliminary acoustic analysis has revealed no overlap in call repertoire between this and other killer whale groups, however it most closely resembles the residents in both call frequency (J.K.B. Ford, pers. comm.) and echolocation frequency (L. Barrett-Lennard, unpublished data). In this study it is shown to be more closely related to the resident and north Atlantic killer whales than to the transient groups.

Northern Residents

The northern residents generally travel in larger, more stable groups than the sympatric BC transients, and they prey principally or entirely on fish. Members of the group are sighted from central Vancouver Island to the southern part of the Alaskan panhandle. The northern residents comprise three "acoustic clans" with different acoustic repertoires (Ford 1991). Genetically, it is closely related to the southern resident group, the offshores, and north Atlantic killer whales, as shown in Figure 1.

Southern Residents

The southern resident group resembles the northern residents in behaviour and diet. It consists of single acoustic clan, with a different call repertoire from each of the northern resident clans (Ford 1991). It is normally sighted in the waters of southern British Columbia and northern Washington State, south of the range of the northern residents, however it is occasionally sighted in waters frequented by the northern residents (G.M. Ellis, unpublished data). It has never been seen to associate with either the northern residents or the BC transients, and is genetically distinct from both

(Hoelzel 1991, L. Barrett-Lennard unpubl. data).

Prince William Sound Residents

This group of killer whales resembles the northern and southern residents in diet and behaviour. Prince William Sound appears to be near the eastern edge of the range of most of the pods in this group, however two pods commonly seen near the north end of the range of the northern resident group (AF and AG) are occasionally seen in Prince William Sound. The fact that some of the Prince William Sound pods match the northern residents and some match the southern residents in mtDNA D-loop sequence suggests that Prince William Sound may have been colonized by whales of both lineages. The data provide no evidence that the Prince William Sound residents are a unique, genetically-isolated population.

The maximum likelihood analysis clearly indicates a common origin for all three transient groups. Thus, we reject the hypothesis that transient-type and resident-type killer whales arose from a common ancestral form more than once. The BC transients and Gulf of Alaska transients likely arose from a common stock relatively recently---indeed, further sampling may prove that the apparent geographic separation between the two lineages is not perfect. The AT1 lineage is clearly derived from a transient ancestor, but beyond that the origins of the group are unknown. It may be an isolated representative of an unsampled population, perhaps in the Arctic or Western Pacific, or may the remnant of a declining lineage. Given its small population size and apparent isolation, it seems unlikely to persist.

The relationships between the two resident groups, the offshores, and the north Atlantic killer whales are not well resolved, except that they clearly cluster independently of the transients. The fact that offshores appear closely related to residents, and that all 3 assemblages, leads us to speculate that members of a "protoresident/offshore" assemblage moved between the Pacific and Atlantic basins after the separation with transients occurred. Much more widespread sampling will be necessary

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before this hypothesis can be evaluated.

Conclusions

1) The residents and transients of Prince William Sound belong to different populations. The mitochondrial DNA analysis presented here supports field observations that the resident and transient assemblages in Prince William sound are distinct populations.

2) *Residents and transients diverged once*. The comparison of mtDNA D-loop sequences between Prince William Sound and British Columbian killer whales indicates that the resident and transient assemblages are separate, long-standing lineages, not evolutionarily re-occurring specialist forms.

2) Separation of BC transients and Gulf of Alaska transients. The evidence that at least two major transient populations inhabit the Eastern Pacific coast has important management implications. Barrett-Lennard et al. (1995) estimated the population of BC transients at approximately 170 individuals. By comparison, only 33 transients have been identified in the Gulf of Alaska. The latter figure may underestimate the true number of transients inhabiting the Gulf, since relatively few surveys have taken place in that region. However, it should certainly be regarded as a small population as far as management is concerned.

3) *Identification of the AT1 group as a genetically distinct group*. Previous to this study, it was reasonable to suppose that the AT1's were simply the transient equivalent of a resident pod, or socially-cohesive group of related killer whales, and that they were likely to interbreed with other transients. The mitochondrial data instead suggests that they are reproductively isolated from other killer whales inhabiting the area. If this is true, their population size is likely too small for viability.

4) Relatedness of Prince William Sound residents to Northern Residents and Southern

Residents

In 1995, the size of the Prince William Sound resident population was approximately 285 individuals (G. Ellis, C. Matkin, pers. comm.). The size of the BC northern resident community is approximately 220 individuals, and there are approximately 95 southern residents. If two presently-unclassified resident pods from south eastern Alaska (71 animals, G.M. Ellis, unpubl. data) are also part of this assemblage, it totals approximately 670 animals. This is probably a large enough population size to be genetically viable in the long term (Lande 1991), although more data on gene flow and genetic population structure needs to be collected before we have confidence in this conclusion.

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