

Exxon Valdez Oil Spill
Restoration Project Annual Report

Comprehensive Killer Whale Investigation

Restoration Project 97012
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

Craig O. Matkin
David Scheel
Graeme Ellis
Lance Barrett Lennard
Harald Jurk
Eva Saulitis

North Gulf Oceanic Society
P.O. Box 15244
Homer, Alaska 99603

January 1998

“The Exxon Valdez Oil Spill Trustee Council conducts all programs and activities free from discrimination, consistent with the Americans with Disabilities Act. This publication is available in alternative communication formats upon request. Please contact the Restoration Office to make any necessary arrangements. Any person who believes she or he has been discriminated against should write to: EVOS Trustee Council, 645 G Street, Suite 401, Anchorage, AK 99501; or O.E.O. U.S. Department of Interior, Washington, D.C. 20240.”

Exxon Valdez Oil Spill
Restoration Project Annual Report

Comprehensive Killer Whale Investigation

Restoration Project 97012
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

Craig O. Matkin
David Scheel
Graeme Ellis
Lance Barrett Lennard
Harald Jurk
Eva Saulitis

North Gulf Oceanic Society
P.O. Box 15244
Homer, Alaska 99603

January 1998

TABLE OF CONTENTS

STUDY HISTORY	6
ABSTRACT	6
KEY WORDS	6
PROJECT DATA	6
CITATION	6
EXECUTIVE SUMMARY	7
INTRODUCTION	9
OBJECTIVES	10
FIELD METHODOLOGY	11
POPULATION STATUS	13
Introduction	13
Methods	13
Photographic Analysis	13
Calculation of Vital Rates	13
Results	14
Resident pods	18
Transient whales	22
Killer Whale Predation	24
Discussion	24
CHANGES IN HABITAT USE AND GIS PRODUCTS	25
Introduction	25
Methods	25
Results	26
Discussion	26
POPULATION GENETICS	33
Introduction	33
Methods	34
Biopsy Samples	34
Mitochondrial DNA Sequencing	34
DNA-based Sexing	35
Microsatellite Analysis	35
Results	35
Biopsy Samples	35
Mitochondrial DNA Analysis	36
Genetic Sex Determination	38
Microsatellite Analysis	40
Discussion	40
Population Structure of Prince William Sound Killer Whales	40
Resident killer whales	40
Transient killer whales	41
Genetic Sex Determination	41
Microsatellite Analysis	42
ENVIRONMENTAL CONTAMINANTS	42
Introduction	42
Results	43
Discussion	46

ACOUSTIC ANALYSIS	46
Introduction	46
Methods	47
Selection of recordings	47
Data analysis	48
Qualitative analysis	48
Quantitative Analysis	49
Results	50
Discussion	53
OVERALL CONCLUSIONS	54
ACKNOWLEDGEMENTS	56
LITERATURE CITED	56
Appendix 1	60

List of Figures

- Figure 1. 1997 NGOS vessel (top) and whale (bottom) tracks; 16
- Figure 2. Number of whales in AB pod and in all other well-documented resident pods, 1984-1997; 19
- Figure 3. Numbers of whales in well-documented resident killer whale pods, 1984-1997; 19
- Figure 4. Average number of AT1 transient group whales identified for years with effort greater than 60 field days; 23
- Figure 5. Encounter rates for resident single pods by month 1984-95; 26
- Figure 6. Encounter rates for 2 resident pods by month 1984-95; 26
- Figure 7. Encounter rates for 3+ resident pods by month 1984-95; 26
- Figure 8. Encounter rates for AT1 transient group by month 1984-95; 28
- Figure 9. Encounter rates for Gulf of Alaska transients by month 1984-95; 28
- Figure 10. Encounter rates for resident single pods by year 84-95; 29
- Figure 11. Encounter rates for 2 resident pods by year 1984-1995; 29
- Figure 12. Encounter rate with 3+ resident pods by year 1984-95; 29
- Figure 13. Encounter rates for AT1 transient group by year 1984-96; 30
- Figure 14. Encounter rate for Gulf of Alaska transients by year 1984-96; 30
- Figure 15. Use of Prince William Sound by resident killer whales by year 1984-1996; 31
- Figure 16. Use of Prince William Sound by resident killer whales by month 1984-1996; 31
- Figure 17. Use of Prince William Sound by transient killer whales by year 1984-1996; 32
- Figure 18. Use of Prince William Sound by transient killer whales by month 1984-1996; 32
- Figure 19. Consensus of 1000 bootstrapped maximum likelihood trees; 38
- Figure 20. Photograph of ethidium bromide-stained agarose gel showing PCR amplification of both the SRY region of Y chromosome and a fragment of mitochondrial DNA as a control; 39
- Figure 21. Acrylamide gel radiograph of ³³P-end-labelled PCR products of a microsatellite locus amplified from resident killer whale DNA; 40
- Figure 22. Mean levels of PCBs and DDTs in resident killer whales; 44
- Figure 23. Two call variants of type AKS 01 produced by AK pod (upper picture) and AD pod (lower picture); 49

List of Tables

- Table 1. Effort by vessels in 1997; 15
- Table 2. Encounters with killer whales by vessel in 1997; 15
- Table 3. Summary of killer whale encounters in 1997; 17
- Table 4. Recruitment and mortalities in Prince William Sound resident pods; 20
- Table 5. Mortality and recruitment rates in Prince William Sound resident pods; 21
- Table 6. Resident pods: number of whales and number of encounters in 1997; 22
- Table 7. Killer whales analyzed for mtDNA D-loop sequences; 36
- Table 8. Distribution of mtDNA D-loop haplotypes; 37
- Table 9. Sexes of individual whales determined by SRY region amplification; 39
- Table 10. Levels of total PCBs in selected groupings of killer whales; 43
- Table 11. Levels of total DDTs in selected groupings of killer whales; 45
- Table 12. Level of significance for student t-tests comparing total PCB and total DDT levels in paired sample groups with unequal variance. (p values); 45
- Table 13. Encounters with recordings of six pods in each year of the study period 1984 to 1994;48
- Table 14. List of all identified call types and variation forms in alphanumerical order; 51
- Table 15. Similarities and differences in call type frequency indices between pods that share a major portion of their repertoires; 52
- Table 16. Summary of remote hydrophone recordings; 52

Comprehensive Killer Whale Investigations

Restoration Project 97012 Annual Report

STUDY HISTORY: The current project was initiated under Restoration Project 95012 and this is the third 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 and a non-reviewed annual report submitted in March 1997. An assessment of the status of killer whales from 1984 to 1992 in Prince William Sound was published (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 (Matkin et al. 1997b) and 1997 (97012a). A journal article describing killer whale movement and distribution has been published (Matkin et al. 1997a). Papers have been journal submitted detailing social structure and genealogy of resident killer whales (Matkin et al. in prep.), and describing feeding habits of resident and transient killer whales (Saulitis et al. in prep.)

ABSTRACT: Monitoring of killer whales (*Orcinus orca*) was continued in 1997 using photo-identification methods. There were two calves recruited and one mortality in AB pod. Nine individuals have been missing from the AT1 transient group since 1990 and one since 1991 and are presumed dead. Resident killer whale use of Prince William Sound, 1984-1996, was examined temporally and spatially using encounter data and GIS techniques. Additional samples taken by biopsy dart were used for genetic and contaminant analysis. Analysis of mtDNA strengthened earlier findings regarding the population structure of killer whales from Prince William Sound and their relationship to killer whales in other areas. Microsatellite analysis of nuclear DNA was initiated. Comparison were made of contaminant levels among different populations, age, and sex classes of killer whales. Analysis delineating acoustic separation of resident pods was initiated and a remote hydrophone installed in Prince William Sound.

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

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

CITATION: Matkin, C.O., D. Scheel, G. Ellis, L. Barrett-Lennard, H. Jurk, and E. Saulitis. 1998. Comprehensive killer whale investigation, *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 97012), North Gulf Oceanic Society, Homer, Alaska.

EXECUTIVE SUMMARY

Killer whales were monitored in Prince William Sound, Alaska with funding from the Exxon Valdez Oil Spill (EVOS) Trustee Council in 1989, 1990, and 1991 (damage assessment) and in 1993 (restoration monitoring). Monitoring was continued in 1995-1997 as part of the EVOS Trustee Council restoration program. The North Gulf Oceanic Society (NGOS) independently maintained a monitoring program in all other years since 1984 (Matkin *et al.* 1994). This report summarizes results of the monitoring of killer whales in Prince William Sound in 1997. The goal of the monitoring has been to obtain identification photographs of all whales in all major resident pods and the AT1 transient group on an annual basis. Photo-identification techniques (after Bigg *et al.* 1990) were used to identify individual whales. The current photographic database includes thousands of frames of film collected from 1984-1997 used to provide individual identifications for each encounter with whales. Vital rates for AB pod and all other frequently sighted resident pods were calculated based on the photographic data and provided in tabular format.

The total number of whales in well-known resident pods other than AB pod has increased from 66 to 88 whales from 1988 through 1997, while AB pod has declined from 36 whales to 24 whales in that same time period. All resident pods have increased since 1984 except AB pod. From 1995 to 1997 AB pod had a net increase of two individuals, due to recruitment of four calves and two mortalities. Although AB pod numbers are again on the upswing it would be premature to predict a recovery of this pod. Part of the pod (AB 25 subgroup) still travels with AJ pod.

Sighting data for the AT1 transient group in 1997 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. In 1996 and in 1997 only six members of the AT1 group were photographed (these were not the same six whales in both years). The rate of encounter with members of this group has also declined. Modeling of resighting data for the individual AT1 group whales supported the hypothesis that the missing whales are dead or have permanently emigrated from Prince William Sound (Matkin *et al.* 1996).

Data on killer whale behavior and predation events were recorded in a standard format during all years of the monitoring program. Vessel tracks and maps of whale movements were also maintained. Data entry into the GIS database has been completed for all NGOS killer whale records from 1984 to 1997, including a total of 1,612 boat-days of search effort and 713 encounters with whales. These data were error-checked for consistency with the original data sheets recorded in the field.

After correcting for search effort (based on kilometers of boat survey per year), we identified four patterns of area use by killer whales, two among resident pods, and two among transient groups. Area use was similar in resident pods AB, AE, AI, and AN, which all tended to use Knight Island passage and Knight passage more than other areas of the Sound. This pattern was different from that of resident pods AJ and AK, which used all areas of the Sound more evenly. The transient groups made relatively common use of the southwest bays and passages. The AT1 group was also biased towards the use of mid- and eastern-Sound waters more than any other group, while Gulf of Alaska (GOA) transients were more frequently found in Montague Strait or just outside the Sound. Despite these differences, Knight Island and Knight passages were among the most used areas for all groups. The dichotomy between residents, in Montague

Straight and Knight Island Passage, and transients, in the narrow bays and passages, reflects dietary preferences, as salmon migrate through Montague Strait and Knight passage, while foraging tactics on pinnipeds appear to require careful searching of areas very close to shoreline, such as in the southwest bays and passages. (See Appendix 1)

A total of four observations of predation were made in 1997. These were primarily predation on coho or silver salmon (*Onchorynchus kisutch*) by resident killer whales in Resurrection Bay. Enhanced coho salmon and chinook salmon (*Onchorynchus tshawtscha*) returns may be responsible for the increased presence of resident killer whales in Kenai Fjords in recent years. A complete account of historic observations of predation are provided in Matkin *et al.* (1997b) and Saulitis *et al.* (in prep).

Biopsy tissue sampling for genetic analysis and contaminant analysis was continued in 1997 using a biopsy dart system and field techniques developed by Barrett-Lennard *et al.* (1996). An additional 29 tissue samples from individually identified killer whales were collected in 1997. Of these, 27 contained sufficient blubber for contaminant analysis. A total of 76 full-sized samples have been collected from resident and transient killer whales.

The entire mitochondrial DNA D-loop region of the newly-biopsied killer whales were sequenced. Analysis of these sequences refined our understanding of the previously-described genetic divergence between resident- and transient-type killer whales, and supported the existence of two genetically-distinct groups of resident killer whales in Prince William Sound. It also indicated that one of the resident groups shares a common lineage with residents from southern British Columbia and northern Washington waters (Southern residents), and the other with residents from northern British Columbia and southeast Alaska (Northern residents). Furthermore, the genetic division of Prince William Sound residents is congruent with strong differences in vocal call repertoires, suggesting a long-standing cultural separation. The genders of all biopsied killer whales that had not been sexed during field observations were determined genetically. Nuclear microsatellite loci were screened for use in paternity and population analysis. Six variable loci were identified. Microsatellite typing of all sampled Prince William Sound killer whales at these loci is currently underway.

An additional 27 samples were analyzed for environmental contaminants at the National Marine Fisheries Service, Environmental Contaminant Laboratory, Seattle in 1997. Analysis of additional samples clarified the much higher in contaminant levels in marine mammal-eating transient than in fish-eating resident killer whales and provided a large enough sample size to statistically examine differences in contaminant levels between particular groups of resident killer whales. This analysis confirmed earlier indications that reproductive status, sex, and genealogy strongly influence contaminant levels in individual whales. Reproductive females had significantly lower levels of contaminants than other groups. First born offspring had the highest levels. Analysis supported the hypothesis that contaminants are passed from mother to offspring during lactation.

During the first year of acoustic analysis, we concentrated on assessing the repertoires of the most frequently encountered resident pods that use Prince William Sound/Kenai Fjords. Recordings made during the time period 1984 to 1994 from six pods; AB, AD (now AD05 and AD16), AE, AI, AK, AN (now AN10 and AN20) were analyzed and 8456 calls were digitized and spectrographically compared. The results showed that these pods have distinct repertoires which can be called pod-specific dialects. Twenty eight call types have been identified so far. According to preliminary results of acoustic similarities the six pods fall into two clusters. The first cluster contains AB, AI, and AN pod, while the second cluster contains AD, AE, and AK pod. Each pod in cluster one uses an average of 11.66 calls (range: 8-15), while each pod in cluster two uses an average of 7.66 calls (range 7-8). Recordings during January and February 1996 from a remote hydrophone located near the connection of Knight Island Passage and Montague Strait revealed the presence of at least two to four pods; AB, AK, and possibly AD and AI.

INTRODUCTION

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

The AB pod and AT1 group appear to have been injured due to the effects of the *Exxon Valdez* oil spill. Although AB pod has shown a net increase since 1995, it is far from recovering to pre-spill numbers. The AT1 group does not appear to be recovering. Numbers of whales in other well-documented resident pods continue to increase. Annual photographic monitoring has been the most effective tool in determination of the recovery status of AB pod and the AT1 group and the status of the entire Prince William Sound killer whale population (Matkin *et al.* 1994). This project continues using photo-identification to monitor changes in resident killer whale pods (including AB pod) and the AT1 transient group in Prince William Sound.

Predation by killer whales may be a factor in the non-recovery of harbor seals in Prince William Sound following the *Exxon Valdez* oil spill. The decline of harbor seals may also be related to the non-recovery of the AT1 group of transient killer whales. At least 300 harbor seals were killed at the time of spill and the harbor seal population continues to decline. Of the two types of killer whales in Prince William Sound, only one, the 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 AT1 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 manuscript detailing feeding behavior has been submitted to Marine Mammal Science (Saulitis *et al.* in review).

This project examined harbor seal predation parameters using historical killer whale sighting and behavioral data in a geographic information system (GIS) framework. Predation of harbor seals by killer whales is considered one probable factor that may limit the recovery of seals. These results can then be incorporated into models of harbor seal population dynamics (project 064, seal trophics). To accomplish this, a geographic information system (GIS) database was designed and the data from 1984 to 1997 entered into a computer from hand-written data sheets. Sighting records provide considerable behavioral information (travel rates, duration of feeding bouts, etc.). Location of encounters and basic behavioral information (resting, feeding, travelling, etc.) are available for each sighting.

It is the goal of the GIS project to provide geographically-referenced analysis of these data to address questions of interest to restoration management, and to examine the distribution of whale groups over time in Prince William Sound. Data analysis is providing detailed demographics and spatial distributions of resident and transient killer whales (Appendix 1).

This project also examined the separation of marine mammal-eating transient whales and fish-eating resident killer whales using behavioral data and genetic analysis. Genetic samples have been obtained from 76 whales. Samples were obtained using lightweight biopsy darts (Barrett-Lennard *et al.* 1996). The genetic analysis has focused on mitochondrial DNA (mtDNA) and the separation of populations. The development of microsatellite loci and genetic sexing of individual whales was an emphasis of the 1997 work. MtDNA evolves quickly, is only passed through the maternal line, and provides a faithful record of female lineages over long periods. MtDNA is considered an appropriate marker for distinguishing well-established populations. Microsatellite analysis will provide further resolution of populations, and detail of killer whale social structure and breeding systems.

Contaminant analysis is being completed on blubber tissue collected simultaneously with the genetic samples. Patterns in contaminant accumulation suggest the importance of reproductive status and genealogy in determining contaminant levels. 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.

Killer whales can be found regularly in Alaskan waters, but only a few locations allow acoustic tracking of unknown animals for group identification and community assessment purposes. The noise in some areas may also interfere with the whales' ability to communicate with each other which may cause avoidance of those areas. Prince William Sound, Alaska is an acoustically pristine area in which tracking of killer whales by calls is possible. Since the mid-1980s systematic field studies on killer whales of this area we have opportunistically recorded killer whale vocalizations while identifying individuals photographically. As a result, a relatively large number of acoustic recordings exist in addition to photo-identification pictures of killer whales. Many of these recordings were made prior to the oil spill in 1989 and this data has provided a basis for comparison of vocalizations before and after the oil spill.

OBJECTIVES

1. To monitor changes in AB pod, the AT1 transient group and the other major resident pods in Prince William Sound.
2. To identify individual whales photographed on a frame by frame basis and complete entry of identification data for 1997 into a photographic database.
3. To complete input of observational data for 1997 into the specially designed GIS system at the Prince William Sound Science Center.
4. To examine changes in habitat use by resident pods in Prince William Sound using GIS techniques and to draft a journal publication (Appendix 1).
5. To examine changes in encounter rates and in whale use rates for southwestern Prince William Sound for 1984-1996.
6. To continue field observations of killer whale behavior and predation.

7. To refine genetic separation of killer whales using mtDNA analysis and design and initiate microsatellite analysis of nuclear DNA for Prince William Sound/Kenai Fjords killer whales.
8. To continue examination of contaminant levels in Prince William Sound/Kenai Fjords killer whales based on an increased sample size.
9. To analyze acoustic data collected from 1984-1997 and determine pod specific killer whale dialects and vocal similarities between members of the same clan.
10. To establish a remote hydrophone in southwestern Prince William Sound and monitor during winter months.

FIELD METHODOLOGY

Most field work for the 1997 photo-identification study was conducted from the R.V. *Lucky Star*, 12.8 m inboard diesel powered vessel which carried a 5m outboard powered console skiff. Photo-identification was conducted from the skiff while acoustic recordings were made from the R.V. *Lucky Star*. The R.V. *Lucky Star* operated primarily in the Kenai Fjords region. In addition, the R.V. *Whale 2*, a 7.9m, live-aboard vessel powered by a 165 hp diesel engine with inboard/outboard drive operated primarily in the Prince William Sound region.

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

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, unless sighting information indicated changes in whale distribution. Whales were found visually, or by listening for killer whale calls with a directional hydrophone, or by responding to VHF radio calls from other vessel operators. Regular requests for recent killer whale sightings were made on hailing Channel 16 VHF. Photographs for individual identification were taken of the port side of each whale showing details of the dorsal fin and saddle patch. Photographs were taken at no less than 1/1000 sec using Ilford HP5, a high speed black and white film, exposed at 1600 ASA. A Nikon 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 and have been placed in a GIS format and used to estimate effort (Matkin *et al.* 1996, 1997b). On these logs the elapsed time and distance travelled were recorded. Vessel track was plotted. Record was made of time and location of all whale sightings and weather and sea state noted at regular intervals.

Specifics of each encounter with killer whales were recorded on standardized data forms that have been used since 1984. These forms were modified in 1995 to improve collection of data for GIS input (Matkin *et al.* 1996). Data recorded included date, time, duration, and location of the encounter. Rolls of film exposed and the estimated number of whales photographed also were recorded. A chart of the whales' trackline during the encounter was completed and the distance travelled by the vessel with the whales calculated. Specific group and individual behaviors (i.e. feeding, resting, travelling, 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 prey of killer whales were made when possible during the 1997 fieldwork. Only events that provided positive evidence of a kill were categorized as predation. Evidence included prey observed in the mouth of the whale, bits of hair or other parts, or oil slicks with bits of blubber. Incidents of harassment of potential marine mammal prey were also recorded. This included instances where evidence was not observed but a kill was suspected or when potential prey exhibited fright or flight response or other strong behavioral reaction to killer whales. Harassment was demonstrated by behaviors such as flipper slapping and lobtailing by humpback whales and fleeing behavior by small cetaceans, pinnepeds, or mustelids. When predation on fish was observed, scales from the site of fish kills were collected and later identified by species. Scales were individually mounted and identifications were made by the fish scale and aging laboratory at the Pacific Biological Station. Fish scales and marine mammal remains were collected with a fine mesh net on an extendible handle (5 m. maximum extension). The pod or group of killer whales and specific individuals present at the kill or harassment incidents were recorded on the encounter data sheets.

Biopsy samples were collected using a pneumatic rifle and custom-designed biopsy darts (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 and aluminum dart (approx. 10 cm long by 1.2cm dia.) was fitted with a bevelled 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, bounced off, and floated with sample contained until retrieved.

The biopsy samples the epidermis, which was heavily pigmented and 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, solvent-washed vials for contaminant analysis.

Acoustic recordings were made using an Offshore Acoustics omnidirectional hydrophone in combination with Sony Walkman professional tape recorder. During the 1997 field season we used a Sony TCD-8 DAT recorder. The hydrophone had a flat frequency response to signals ranging from 100Hz to 25 kHz. The tape and DAT recorders showed a flat response to signals up to 15kHz.

POPULATION STATUS

Introduction

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

Methods

Photographic Analysis

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

The alphanumeric code used to label each individual was based on Leatherwood et. al. (1984) and Heise *et al.* (1992). 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.

Calculation of Vital Rates

Most 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, Matkin *et al.* in prep). 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 re-identify calves.

If a whale from a resident pod is not photographed swimming alongside other members of its matrilineal group during repeated encounters over the course of the summer field season it is considered missing. If it is again missing during the repeated encounters in the following summer season it is considered dead. No individual resident whale missing during repeated encounters with its maternal group over the course of a summer season has ever returned to its pod or appeared in another pod in all the years of research in Canada and the United States (Bigg *et al.* 1990, Matkin *et al.* 1994, Matkin *et al.* 1997b). 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 a given year

NP = number of whales present in a pod at
end of the previous year

NR = number of calves recruited to
0.5 years in a pod in a given year

then: Mortality rate = NM/NP and Reproductive rate = NR/NP

If the year a mortality or recruitment occurred could not be determined it was split between the possible years. A mean weighted mortality and reproductive rate for all pods for all years was determined by pooling the data.

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

Sighting data for individual transient killer whales was recorded. The cumulative number of different AT1 individuals was plotted against effort (days in the field) for the 1997 season and compared with similar data averaged for 1984-89 and 1990-1995. AT1 whales that had not been resighted for five or more years were suspected dead.

Results

The *Lucky Star* completed 57 days and the *Whale2* completed 35 days of dedicated killer whale surveys and sampling. The *Whale 1*, completed an additional 33 survey days with a primary objective of humpback whale identification. A total of 126 survey days (LOG entries) were entered in the GIS database for 1997 (Table 1). Researchers travelled approximately 10,597 km over a period of 1099 hours. There was considerable effort in both the Prince William Sound and Kenai Fjords region (Figure 1.)

Table 1. Effort by vessels in 1997.

Vessel	#Vessel days	Distance (km)	Time (hours)
<i>Lucky Star</i>	57	4,947.7	583.9
<i>Whale 1</i>	33	2,800.2	251.1
<i>Whale 2</i>	35	2,659.2	257.7
<i>Glacier Expl</i>	1	190.06	6.2
Totals	126	10,597.2	1,098.9

Killer whales were encountered on 50 occasions in 1997 (Table 2). Researchers spent 205.3 hours travelling 1,365.7 km with killer whales.

Table 2. Encounters with killer whales by vessel in 1997.

Vessel	#Encounters	Time w/ whales	Km w/ whales
<i>Lucky Star</i>	33	152.4	998.7
<i>Whale 1</i>	2	2.7	23.9
<i>Whale 2</i>	13	49.3	337.7
<i>Glacier Expl</i>	2	0.8	5.4
Totals	50	205.3	1,365.7

In 1997 there were forty encounters with resident pods and one encounter with a new probable resident pod. There were six encounters with the AT1 transient group, two encounters with Gulf of Alaska transients and one encounter with a whale unidentifiable from the photographs (Table 3).

Encounter rates were much lower in PWS than in Kenai Fjords. In Kenai Fjords there were 35 killer whale encounters for 44 vessel days (0.79 encounters/day) and in Prince William Sound there were 11 killer whale encounters for 80 vessel days (0.14 encounters/day). The encounter rate for Kenai Fjords in 1997 is higher than any rate recorded for Prince William Sound from 1984 through 1996. The encounter rate for Prince William Sound in 1997 is lower than for any other year from 1984 through 1996. All encounters of three or more resident pods ("superpods") occurred in late July, August, September and October (Table 3).

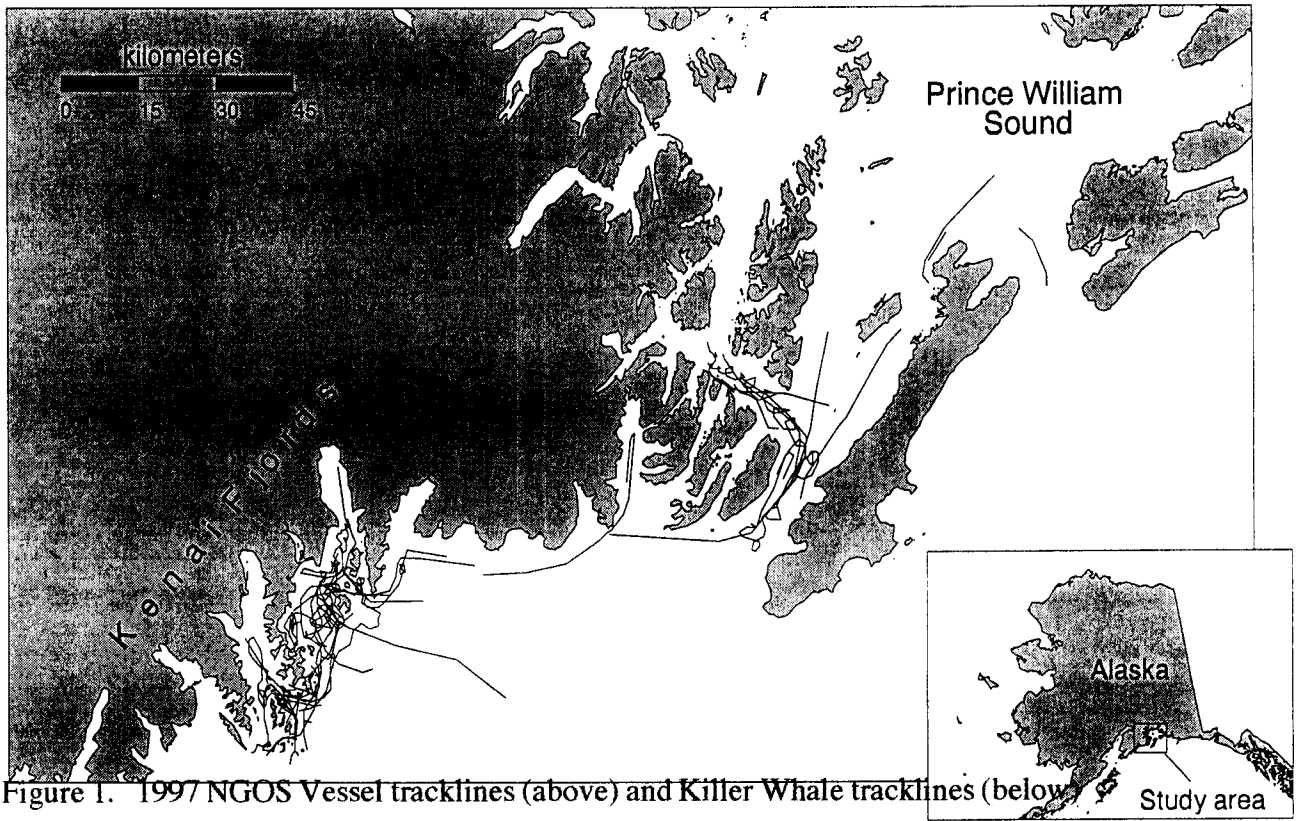
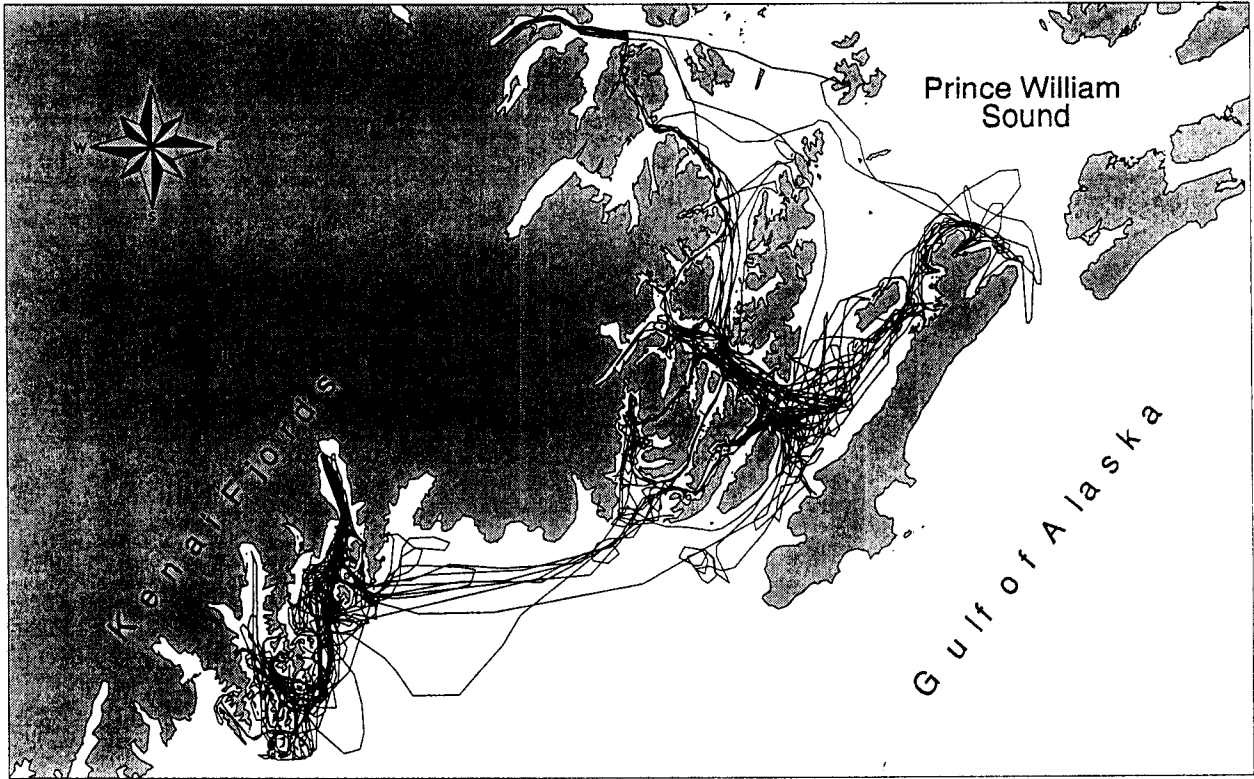


Figure 1. 1997 NGOS Vessel tracklines (above) and Killer Whale tracklines (below)

Table 3. Summary of killer whale encounters in 1997.

#	DATE	Begin Location	End Location	Pods	#Whales
1	4/12/97	1 mi NW Stockdale	2 mi N Stockdale Hbr	AE,AI	22
2	4/14/97	1 mi E Rocky Bay	4 mi E Schooner Rk	AE	15
3	4/15/97	off Stockdale Hbr	3 mi E Rocky Bay	AK	5
4	4/21/97	N end Main Bay	1.5 mi NW Nellie Juan It	AK	5
5	5/17/97	off Chat Is	Chiswell Is	AD16+?	28
6	5/20/97	off Chat Is	S end Chiswells	AK+?	20
7	5/20/97	N end Notoa Is	Off No Name Is	AT60	2
8	5/20/97	Agnes Cove	Agnes Cove	AD5	9
9	5/21/97	N end Agnes Bay	E side Rugged Is	AK	10
10	6/11/97	1 mi N Agnes Cove	2 mi E Porcupine Cove	AD5	9
11	6/16/97	N end P of W Psg	N end Latouche Is	AE,AI	22
12	6/17/97	2mi S of Pleiades	4 mi SW Sleepy Bay	AE,AI	22
13	7/9/97	Pleiades	N end P of W Psg	?	1
14	7/17/97	2 mi S Chat Is	inside Chat Is	AK,AD16	17
15	7/19/97	Barwell Is	8 mi E Barwell Is	AK,AD16	17
16	7/21/97	Needle	Needle	AT60	2
17	7/22/97	1 mi S Toe Pt	2 mi SW Cape Aialik	AT1	4
18	7/22/97	1 mi SE Barwell Is	4 mi E Killer Bay	AG,AX	47
19	7/23/97	off Pony Cove	NE Corner Chat Is	AK	10
20	7/25/97	1 mi W Cliff Bay	59 45' / 149 05'	AK,AD5,AG,AX	66
21	7/26/97	Cape Puget	1 mi N Shelter Bay	AD5,AG,AX+?	60
22	7/27/97	2 mi W Green Is	2 mi SW Hanning B.	AD5,AG,AN10,AI,+?	60
23	7/28/97	1 mi SW Hanning Bay	bet Green/Mont Is.	AE	10
24	8/1/97	2 mi N Sleepy Bay	3 mi S Pt Grace	AB,AJ,AI,AD16,AE+?	95
25	8/5/97	1 mi W Caines Head	1.5 mi NW Marys B.	AN10	18
26	8/6/97	1 mi W mid pt Fox Is	4 mi E Cape Res	AN10	18
27	8/6/97	2 mi NW 3 Hole Bay	3 mi S Cape Aialik	AT1	5
28	8/7/97	2 mi SW Mary's Bay	2 mi SE Holgate Arm	AN10,AN20	27
29	8/9/97	1 mi W Cliff Bay	mouth of Holgate Arm	AT1	2
30	8/13/97	3mi S Pt Helen	bet P of W Psg/Long Ch	AN10	18
31	8/14/97	off Auk Bay	3 mi S Cape Fairfield	AJ,AB	61
32	8/14/97	W side Rugged Is	0.5 mi S Rugged Is	AT1	3
33	8/14/97	NW corner Rugged Is	bet Rugged Is and Bear	AK	10
34	8/16/97	2 mi SE Chat Is	Sunny Cove	AK	10
35	8/17/97	2 mi N Pilot Rk	2 mi S Matushka Is	AW	21
36	8/18/97	mouth P of W Psg	off Needle	AB	24
37	8/18/97	0.5 mi W Chiswell Is	2 mi NW Hive Is	AK	10
38	8/19/97	Toe Point	1.5 mi E E side Harbor Is	AI	7
39	8/19/97	4 mi E Notoa Is	5 mi E Notoa Is	AK	10
40	8/24/97	2 mi SW Sew. shipyard	1.5 mi E Barwell	AI,AJ	44
41	8/24/97	off Barwell Is	2 mi N Chevall	AN10	18
42	8/25/97	2 mi N Harbor Is	2 mi N Harbor Is	AN10	18
43	8/26/97	off Thumb Bay	1 mi S Shipyard	AT1	3
44	8/27/97	NW corner Rugged Is	1.5 mi N Pilot Rk	AK,AD16	17
45	9/11/97	2.5 mi E Calisto Head	2 mi S Cheval	AB,AJ,AN10	79
46	9/12/97	2 mi N Fox Is	2 mi SW Mary's Bay	AB,AJ,AN10,AI	87
47	9/13/97	Toe Point	3 mi W S end Mat. I.	AB,AJ,AK,AX,AD16	98
48	9/14/97	1 mi N Toe Point	2 mi N Toe Point	AJ	37
49	9/14/97	2 mi S Cheval Is	3 mi S Cheval Is	AB	24
50	10/11/97	2 mi SW Mary's Bay	2 mi W Sunny Cove	AB,AJ,AN10,AI	87

Kenai Fjords: 35 encounters/44 vessel days Prince William Sound: 11 encounters/80 vessel days

Resident pods

The total number of whales in well-known resident pods other than AB pod has increased from 66 to 88 whales from 1988 through 1997, while AB pod has declined from 36 whales to 24 whales in that same time period (Figure 2). All resident pods have increased since 1984 except AB pod (Figure 3).

From 1995 to 1997 AB pod had a net increase of two individuals, due to recruitment of four calves and two mortalities. The single mortality in 1997 (to be confirmed in 1998) was AB3, an adult male in the AB10 subpod. At the beginning of the study in 1984, AB3 was a maturing male (about years 18 years of age) and his estimated age at death was 31 years. His fin collapsed at the time of the oil spill. He was the final remaining member of a matrilineal group that consisted of 9 individuals prior to the EVOS; that maternal line is now extinct. Two new calves, AB52 born to AB33 and AB53 born to AB27, were recruited in 1997. For both AB33 and AB27 these were first calves. Although AB27 was estimated to have been at least ten years of age (by appearance) in 1984, she did not produce a viable calve until 1996/97 at an estimated 23 years of age. AB 33 was estimated to be 16 years of age in 1996/97. Average age of first successful reproduction is about 15 years (Olesiuk *et al.* 1990).

AB pod was encountered on eight occasions; seven were multi-pod encounters with AJ pod also present. All three AB subpods (AB17, AB25 and AB10 subpods) were accounted for in these seven encounters. In the one single pod encounter with AB pod the AB25 subpod was not present and we suspect it that subpod still travels with AJ pod.

A total of two calves (AJ40 born to AJ3 and AN54 born to AN10) were recruited into the other five well-known resident pods in 1996/97 (Table 4). There were two mortalities in these pods, AK11 and AN49 (to be confirmed in 1998). Annual mortality and recruitment rates were calculated by pod and are listed in Table 5. Two additional calves were born in late season and are listed as 1997/98 calves and will be considered recruited if present next spring. These late season calves were AJ41, born to AJ4 between September 13 and October 11, and AI8 born to AI3 between August 24 and September 12. The cow, AI3, has not recruited a calf in 13 years.

We encountered members of 12 different resident pods in 1997 (Table 6) and photographed a total of 230 resident or probable resident killer whales. Nineteen of these whales were in mixed assemblages and could not be attributed to pod although nine had been photographed previously by Dahlheim (1994) west of Kenai Fjords. An additional new pod of 21 whales (named AW pod), was classified only as probable residents because they were not associated with other known resident whales and we did not obtain genetic material or acoustic recordings to confirm their affiliation.

Figure 2. Number of whales in AB pod and in all other well-documented resident pods, 1984-1997

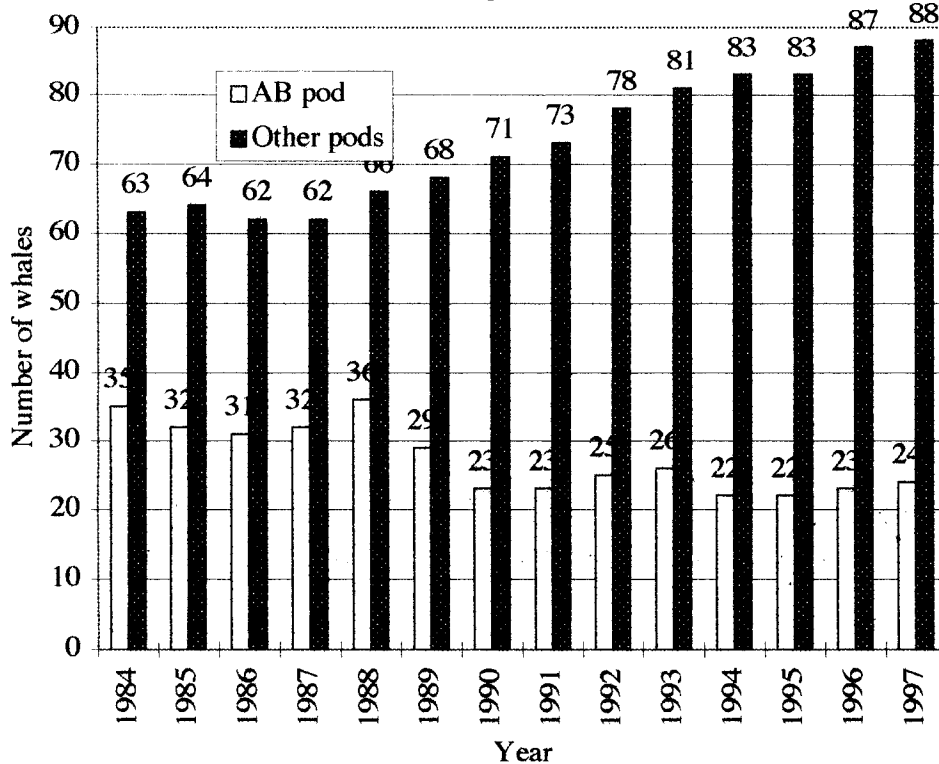


Figure 3. Numbers of whales in well-documented resident killer whale pods 1984-1997

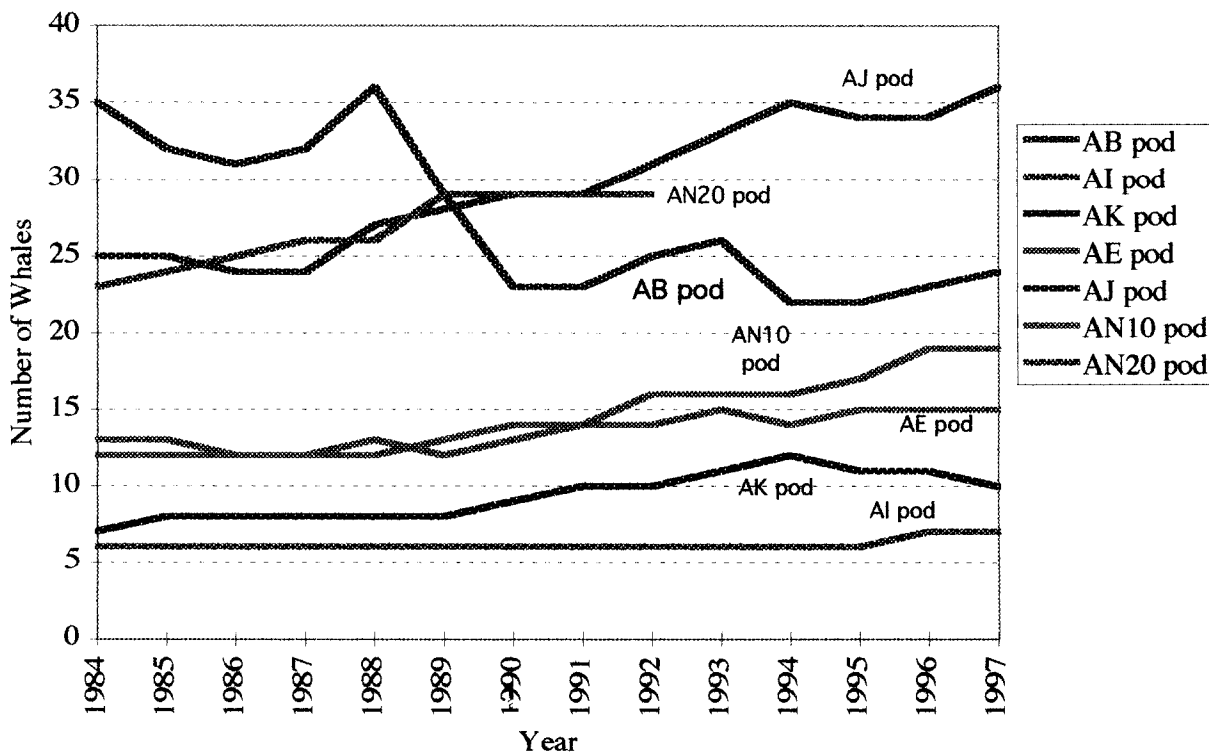


Table 4 Recruitment and mortalities in Prince William Sound resident pods.

POD	Recruitment in Prince William Sound Resident Pods				{ whale number(mothers number)}	
	AB	AI	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) 42(32)			15(10)	26(22),27(20)	40(35)
	43(17), 44(22)				28(24)	
89/90			10(2)	18(11)	30(3)	
90/91	45(16)		11(6)			45(35)
91/92	46(25),47(32)				31(24),32(22) 33(13)	46(10),47(11)
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				20(2)		49(11)
95/96	50(26),51(25)	7(4)			39(13)	50(35),51(12)
96/97	52(33), 53(27)				40(3)	54(10)
POD	Mortalities in Prince William Sound Resident Pods				{by whale number}	
	AB	AI	AK	AE	AJ	AN10
84/85	9,15,34-			8-		
85/86	1,7,12-		5-	4-	23-	
86/87	28-					6-
87/88	6-			7-		
88/89	13,18,21,23,30,31,37			12-		2-
89/90	8,19,20,36,42,44					
90/91	29-					
91/92						
92/93					5-	5-
93/94	2,16,38,41,48			13-	11-	
94/95			4-		6-	
95/96	4-					
96/97	3*		11*			49*
	*to be confirmed in 1998					

Table 5. Mortality and recruitment rates in Prince William Sound resident pods.

Recruitment rates in Prince William Sound Resident Pods							
	AB	AI	AK	AE	AJ	AN10	All other than AB
84/85	0	0	14.3	7.7	0	0	3.2
85/86	6.3	0	12.5	0	0	0	1.6
86/87	6.4	0	0	0	0	8.3	1.6
87/88	15.6	0	0	8.3	12.5	8.3	8.1
88/89	0	0	0	15.4	3.7	7.7	4.5
89/90	0	0	12.5	7.7	3.4	0	4.4
90/91	4.3	0	11.1	0	0	7.7	2.8
91/92	8.7	0	0	0	10.3	14.3	6.8
92/93	4	0	10	7.1	9.4	0	6.8
93/94	3.8	0	9.1	0	5.9	6.7	4.9
94/95	0	0	0	7.1	0	6.3	2.4
95/96	9.1	16.7	0	0	0	11.8	7.9
96/97	8.6	0	0	0	5.9	5.2	3.4
	AB	AI	AK	AE	AJ	AN10	All other than AB
84/85	8.6	0	0	7.7	0	0	1.6
85/86	9.4	0	12.5	7.7	4	0	4.7
86/87	3.2	0	0	0	0	8.3	1.6
87/88	3.18	0	0	8.3	0	0	1.6
88/89	19.4	0	0	8.3	0	7.7	3
89/90	20.7	0	0	0	0	0	0
90/91	4.31	0	0	0	0	0	0
91/92	0	0	0	0	3.4	0	0
92/93	0	0	0	0	0	6.3	2.5
93/94	19.2	0	0	6.7	0	0	2.4
94/95	0	0	8.3	0	2.8	0	2.4
95/96	4.5	0	0	0	0	0	0
96/97	4.3	0	9	0	0	5.2	2.3
# in pod84/97	[35/23]	[6/7]	[7/10]	[13/15]	[25/36]	[12/19]	[63/88]

Table 6. Resident pods: number of whales and number of encounters in 1997.

Pod	#Whales	#Encounters
AB	24	8
AJ	38	8
AG	27	4
AN10	19	10
AN20	9*	1
AI	8	9
AE	15	6
AK	10	14
AD16	7	6
AD5	13	6
AX	20**	4
AW [^]	21 [^]	1
Unclassified	19	1

* only part of pod photographed, 31 total attributed to this pod

** only part of pod(s) present. 70 total whales attributed to this pod, probably 2 or more pods

[^] probable residents, not yet linked by association,acoustics or genetics with other resident whales

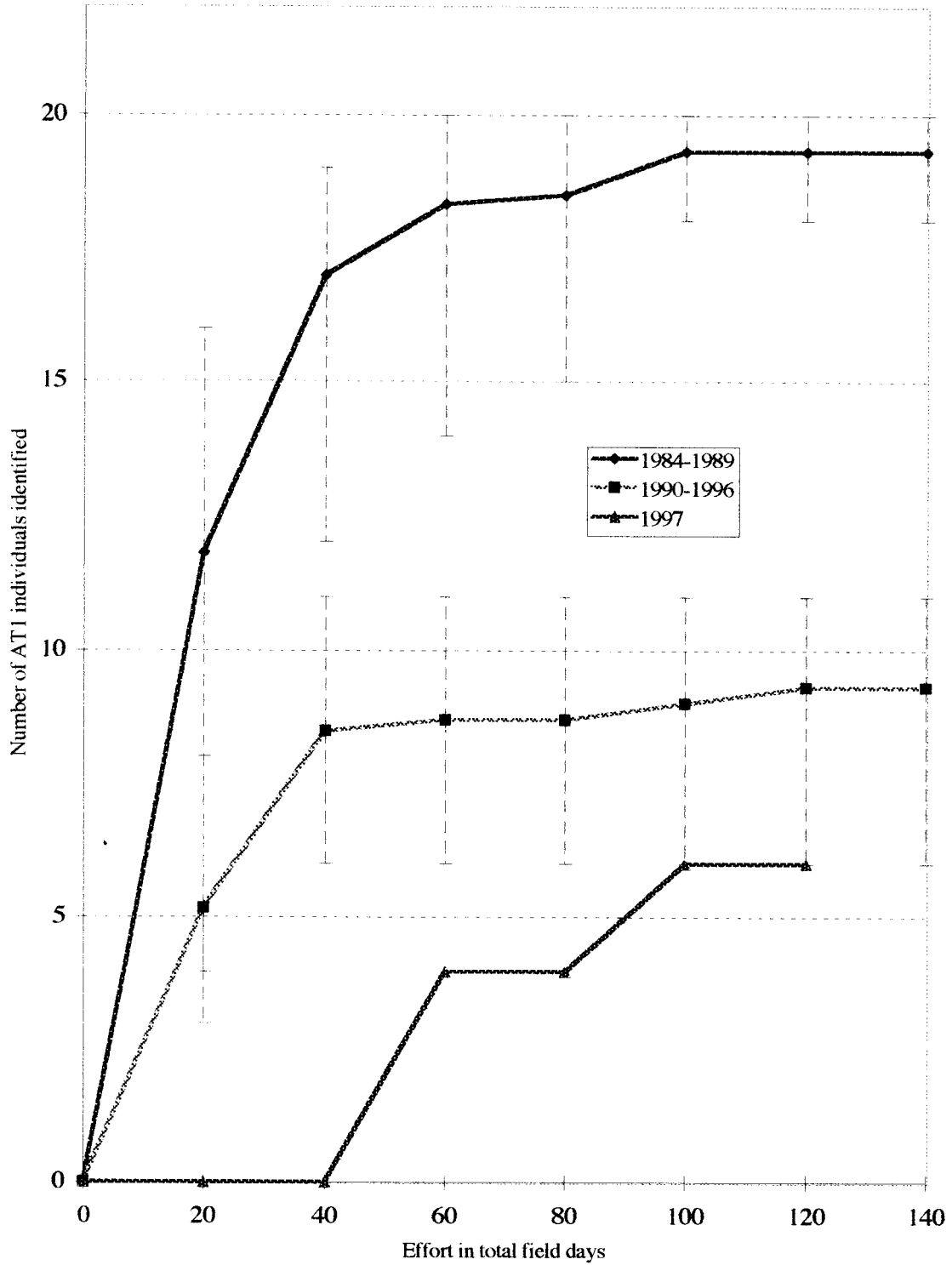
In addition to the pods observed this season, pods AS (approximately 20 whales) and AY (approximately 11 whales) and AF (approximately 46 whales) had been delineated in previous years by examination of photographs but were not observed in 1997. Over the years we have also photographed another 88 identifiable individuals that have not been attributed to pods. The current minimum estimate of resident killer whales that use the Prince William Sound /Kenai Fjords region at least occasionally is 446 whales. This figure was developed using photographs of individuals taken between 1984-97 and subtracting known mortalities. It does not include the probable resident AW pod (21 whales). Of the whales included in this total, two pods, AG and AF, which total 80 whales also have been photographed in southeastern Alaska (Dahlheim *et al.* 1997) and it is suspected they center their range in that region (Matkin *et al.* 1997). Another 20 whales in our total were also photographed by Dahlheim (1997) in killer whale population surveys from Kenai Fjords westward to the eastern Aleutians.

Transient whales

A total of six of the original 22 AT1 group whales were photographed during six encounters in 1997. These were AT1, AT2, AT3, AT4, AT13, and AT17. There were no encounters with these whales in Prince William Sound. Members of the AT1 group were observed only in Kenai Fjords region. Eleven whales in the AT1 group have been missing for six years or more and are considered dead. Since 1989, the number of AT1 individuals identified annually has been 12 or less despite a field effort that exceeded 200 vessel days in 1990 and 1991 and 120 days in 1997 (Figure 4). There were no new calves identified in 1997 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. In 1997 the individuals sighted per effort was below the average for both 1990-1995 and for 1984-1989 (Figure 4).

Figure 4. Average number of AT1 transient group whales identified for years with effort greater than 60 field days (error bars = range)



Both before and after 1989 there was an initial high rate of discovery of non-photographed AT1 individuals in the first 60 days of each field season followed by a sharp reduction of new whale discoveries despite repeated encounters with AT1 whales. In 1997 there was an atypically low rate of discovery of unphotographed AT1 whales during the entire season. This was despite 10 days of field effort in April when historically there has been a high rate of encounter with AT1 whales and a total field effort in 1997 that exceeded 120 days.

Killer Whale Predation

A total of four salmon scale samples were collected in 1997 from the sites of fish kills by resident killer whales. Two were collected in Resurrection Bay from coho salmon (*Onchorhynchus kisutz*) that could be identified by observation at time of the kill. The samples await identification at the scale analysis laboratory at the Pacific Biological Station in Nanaimo, British Columbia. Only resident killer whales were observed preying on fish. There were no observations of predation on marine mammals in 1997.

Discussion

Although there has been a net gain of two individuals in AB pod since 1995, the changes in social structure and reduction in the number of reproductive females in the pod (Matkin *et al.* 1994, Matkin *et al.* 1997) make it difficult to project a long-term recovery. We are encouraged by the recruitment of new calves by AB27 and AB33, females that had not previously produced calves. There are now eight reproductive females (whales that have produced calves in the past ten years) in AB pod. However, we suspect that two of these whales, AB14 and AB17 may be near the end of their reproductive lives, not having produced calves for nine years. The single mortality in 1997, AB3, was a mature male of uncertain age, but estimated to be about 31 years of age based on his appearance at the beginning of study (1984). His death may be related to age/and or health conditions. His dorsal fin collapsed immediately following the *Exxon Valdez* oil spill and dorsal fin collapse may be related to poor health (Matkin *et al.* 1994). The mean life expectancy for male killer whales in the inside waters of Washington State and British Columbia was 29.2 years (Olesuik *et al.* 1990). However, because he had lost all other members of his matrilineal group, including his mother, since the oil spill, we suspect that social factors were involved in his death. In the past two years he had often travelled slowly and separately from the rest of the pod. He was last seen in February 1997 in Resurrection Bay and was travelling apart from other whales in AB pod at that time. His death will be confirmed if he is again missing in 1998.

In seven of eight encounters with AB pod in 1997, the entire pod was travelling with AJ pod in the Kenai Fjords region. The AB25 subpod was not present in the one encounter when AJ pod was absent and we suspect it still travels with AJ pod. This is the fifth consecutive year that this has been observed. There is no precedent for a resident pod subgroup joining another pod on an extended basis (Matkin *et al.* 1994, Bigg *et al.* 1990). Again, this may be a result of the breakdown of social bonds that held the subgroups within AB pod together prior to the oil spill.

Extending the field season into April and May did not result in the intended effect of producing additional encounters with the AT1 transients. We are increasingly convinced that at least 11 of the original 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 to be genetically distinct from all other pods and groups sampled by mtDNA analysis, is headed for extinction.

CHANGES IN HABITAT USE AND GIS PRODUCTS

Introduction

Changes in habitat use during the 1984-1996 period in Prince William Sound were examined by (1) using spatial analytical techniques (GIS), (2) comparing changes in rates of killer whale encounters, and (3) comparing changes in numbers of whales encountered per day.

The GIS generated products developed in 1997 describe the distribution of resident and transient groups over time and area and are presented in Appendix 1 "Distribution of Killer Whale Pods in Prince William Sound, Alaska Over a Thirteen-Year Period". In this paper thirteen years of encounter data (1984 - 1996) were used to examine killer whale distribution within the Sound. The Knight Island region and Montague Strait were among the most used areas for all groups. However, four distinct patterns of area use were identified among resident pods and transient groups. A dichotomy in distribution found between residents, which used the open waters of Montague Strait and Knight Island Passage, and transients, more typically found in the narrow bays and passages, probably reflected dietary preferences. Resident pods AB, AE, AI, and AN were distinct in area use patterns from resident pods AJ and AK. Transient AT1 group was distinct from Gulf of Alaska transients. The different patterns of use among resident pods did not fall out exactly along pod lineages as reflected by haplotype and vocalization data. The reasons for these distinct patterns of area use are currently unclear.

In addition to GIS analysis products, differences in encounter rates with resident and transient whales from 1984-1995 in Prince William Sound were compared by month and by year graphically and statistically. Finally, changes in rate of use of Prince William Sound by resident and transient killer whales were examined graphically and statistically by comparing the number of whales encountered per field day by month and by year for 1984-1996.

Methods

Five categories were used in analysis of killer whale encounter rates. Resident pod encounters were divided into three groups 1) Single pod encounters, 2) two pod encounters and 3) three or more pod (multi-pod) encounters. Transient killer whale encounters were separated into 1) AT1 group encounters and 2) Gulf of Alaska (GOA) transient encounters. The AT1 transients and GOA transients are genetically distinct and we have never seen them in association. The groupings were examined for changes by month (April through September) for all years (1984-1995) and by year for all months. Numbers of encounters were transformed with an arc-sine transformation and the significance of changes in encounter rates by month and by year examined with analysis of variance (ANOVA) procedures. The Tukey-Kramer Honestly Significant Difference procedure was used for all pair wise comparisons after initial tests indicated significance at $\alpha = 0.05$ (Sokal and Rohlf 1995). The rate of use of the Sound by killer whales was also measured by the number of whales encountered per field day (whale days). Only two categories were examined, total resident whale days and AT1 transient group whale days. Total whale days for the Gulf of Alaska transients was judged insufficient for meaningful comparisons. Both categories were compared by months for all years (1984-1996) and by year for all months (April through September). Comparisons were made graphically and using analysis of variance (ANOVA). Between year and between month differences were examined using Tukey HSD test for multiple comparisons where ANOVA significance was determined. For all statistical tests, p values between 0.1 and 0.05 were considered marginally significant, p values from 0.05 to 0.01 were considered significant, and values below 0.01 were considered highly significant.

Results

Over the primary months of the field season (April-September), differences in resident killer whale single pod encounter rates were highly significant (ANOVA $p = 0.005$, Figure 5). Tukey-Kramer tests indicated they were significantly greater in August than in June and July and significantly greater in September than in June or July. There was not a significant difference for encounter rates with two resident pods among months (ANOVA $p = 0.02$), although there was a steady increase in rates from April to September (Figure 6). Differences in encounter rates for three or more resident pods among months were highly significant (ANOVA $p = 0.001$, Figure 7) Tukey-Kramer tests indicated rates were significantly greater in August than in May, June, or July and significantly greater in September than in May, June, or July. There was not a significant difference in encounter rates for the AT1 transient group by month, although the rates declined from April to June. September is the month with the lowest rate (ANOVA $p = 0.20$ Figure 8). There was no significant difference in encounter rates by month for the GOA transients, however, sample size was very small (ANOVA $p = 0.37$, Figure 9).

There was not a significant difference in resident pod encounter rates for single pods (ANOVA $p = 0.60$, Figure 10) or for two pods (ANOVA $p = 0.60$, Figure 11) by year for 1984-1995. There was a marginally significant difference (ANOVA $p = 0.055$, Figure 12) for three or more resident pods. There were no clear trends in resident pod encounter rates over the years except a decline in multi-pod encounters since 1987. There was no significant difference between pairs of years for any of the resident pod groupings using the Tukey-Kramer test. There was also no significant difference in transient AT1 (ANOVA $p = 0.29$, Figure 13) or GOA transient (ANOVA $p = 0.50$, Figure 14) encounter rates over the years, although AT1 encounter rates were higher in 1988 and 1989 than in other years.

Comparisons of the rate of use of the southwestern Sound by resident whales per field day were marginally significant over years (ANOVA $p = .10$). There was a clear downward trend in use over the years, with peak use occurring prior to the oil spill, a sharp decline at the time of the spill in 1989, followed by a slight recovery in 1992 and 1993 and a decline thereafter (Figure 15). Differences in the rate of use by month (all years included) was highly significant (ANOVA $p = .002$). Rates were significantly greater in September than in any other month although they increased from July through September (Figure 16).

The rate of use by AT1 transient whales per field day declined significantly over the 1984 to 1996 period (ANOVA $p = 0.02$). The downward curve was not smooth, but had significant depressions in 1987, 1991 and 1994 (Figure 17). Although April had the highest rate of encounter with AT1 transient whales (Figure 18), there was not a significant difference in rate of use by months (ANOVA $p = 0.51$)

Discussion

Statistical analysis corroborates the subjective observations that encounter rates with resident pods from 1984 to 1995 increased during late July, August, and September in particularly in the southwestern Prince William Sound. During our 1997 fieldwork, this was also the trend in the Kenai Fjords region. In 1997 killer whale encounter rates in the Sound declined dramatically below historic levels and monthly trends were unclear. Analysis also supports our observations that multi-pod encounters (3 or more pods) were most common in August and September (Matkin *et al.* 1997a). In the Kenai Fjords region in September 1997 we observed multi-pod aggregations of resident pods that numbered nearly 100 individuals and occupied the area on a daily basis. These type of aggregations, although common in Prince William Sound in the 1980s had not been observed in that area for several years. Although not statistically significant, there has been an irregular decline in the single resident pod encounter rate since the late 1980's. There has also

Figure 5. Encounter rates for resident single pods by month 1984-95
 Arcsine transformed ANOVA $p=.005$

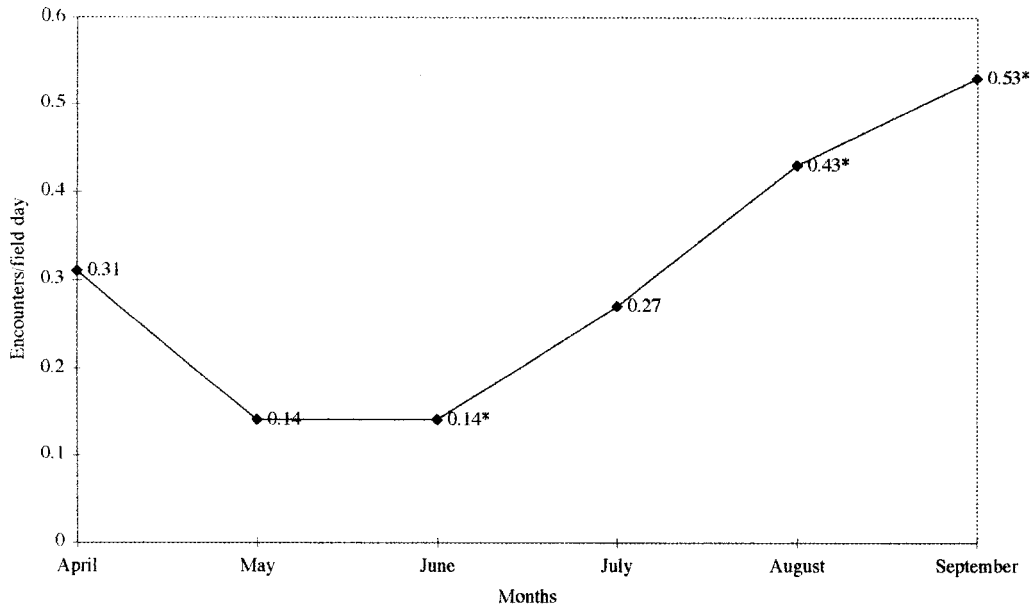


Figure 6. Encounter rates for 2 resident pods by month 1984-95
 Arcsine transformed ANOVA $p = .02$

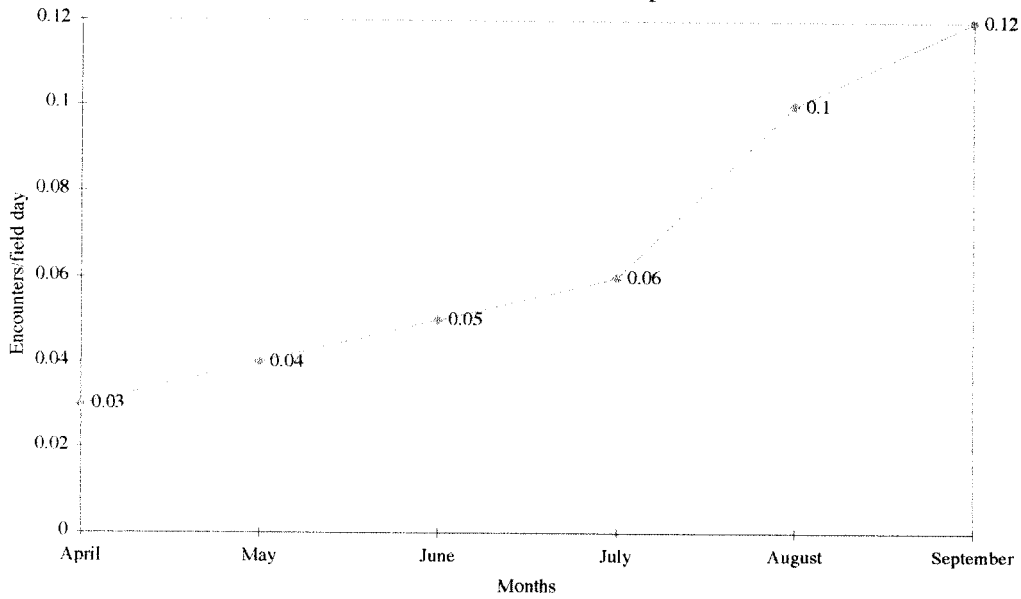


Figure 7. Encounter rates for 3+ resident pods by month 1984-95.
 Arcsine transformed ANOVA $p= .001$

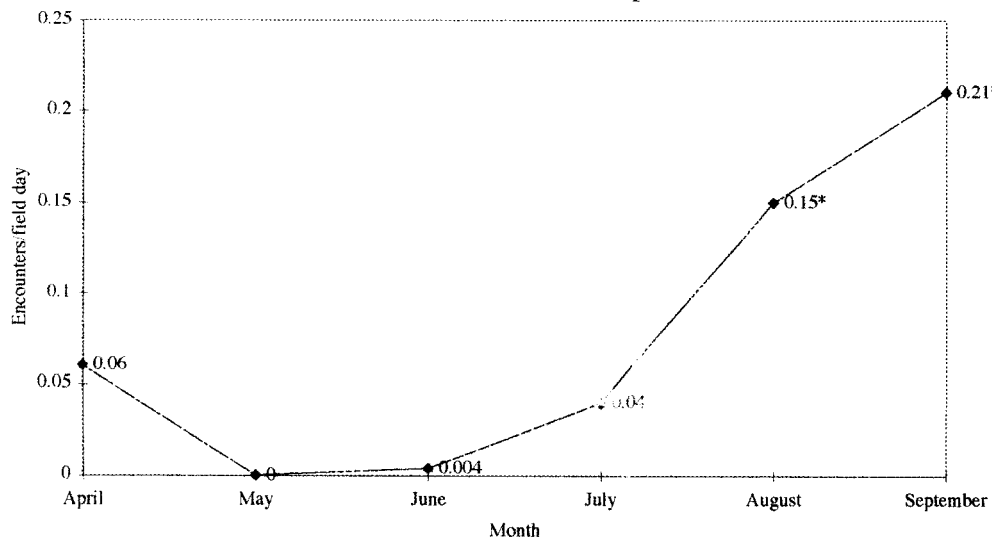


Figure 8. Encounter rates for AT1 transient group by month 1984-95
Arcsine transformed ANOVA $p = .20$

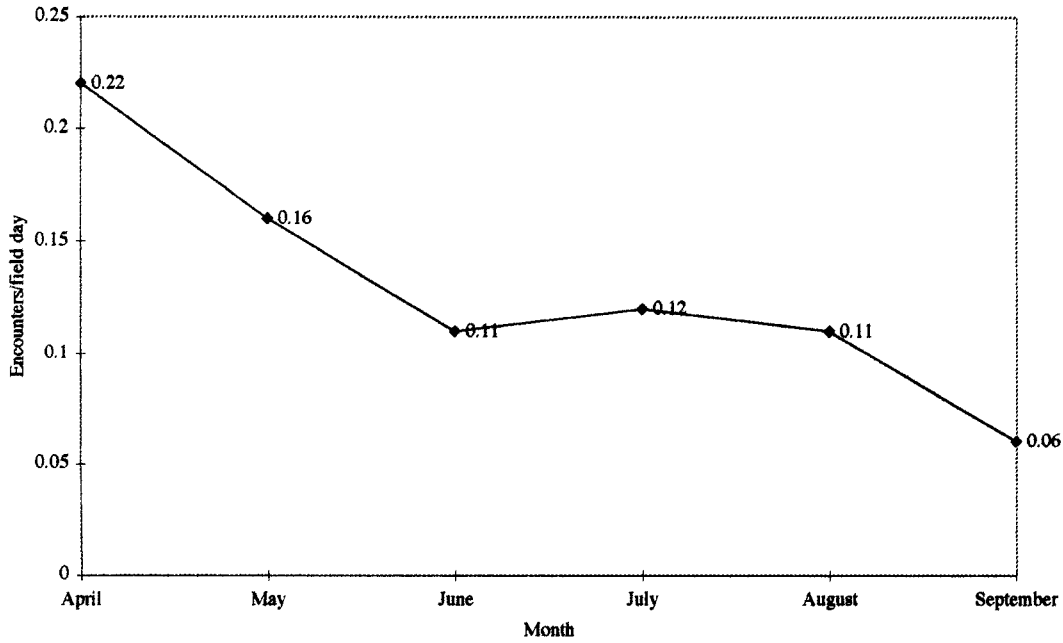


Figure 9. Encounter rates for Gulf of Alaska transients by month 1984-95
Arcsine transformed ANOVA $p = .37$

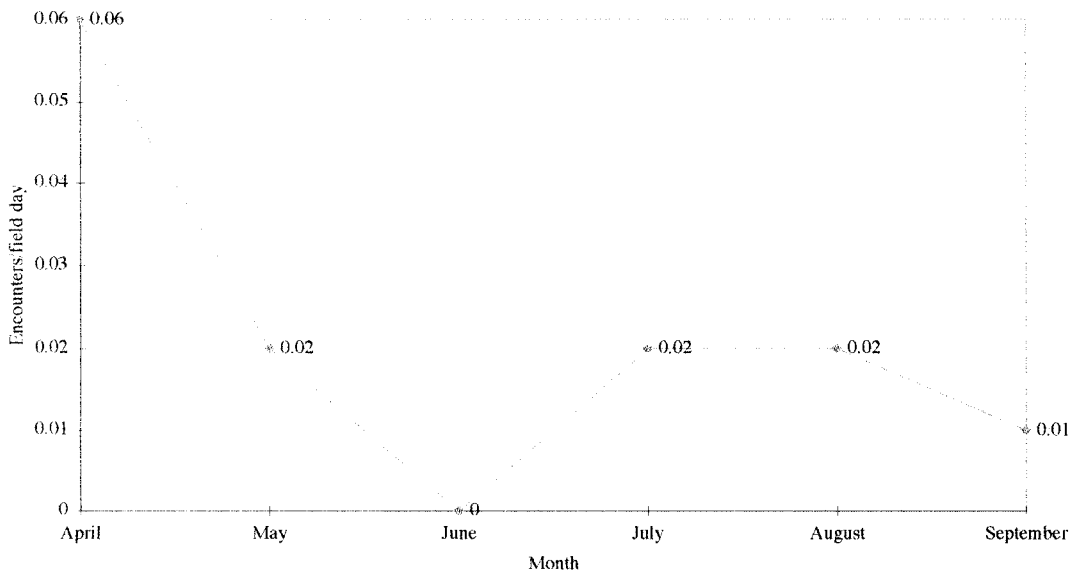


Figure 10. Encounter rates for resident single pods by year 84-95
 Arcsine transformed ANOVA $p = .60$

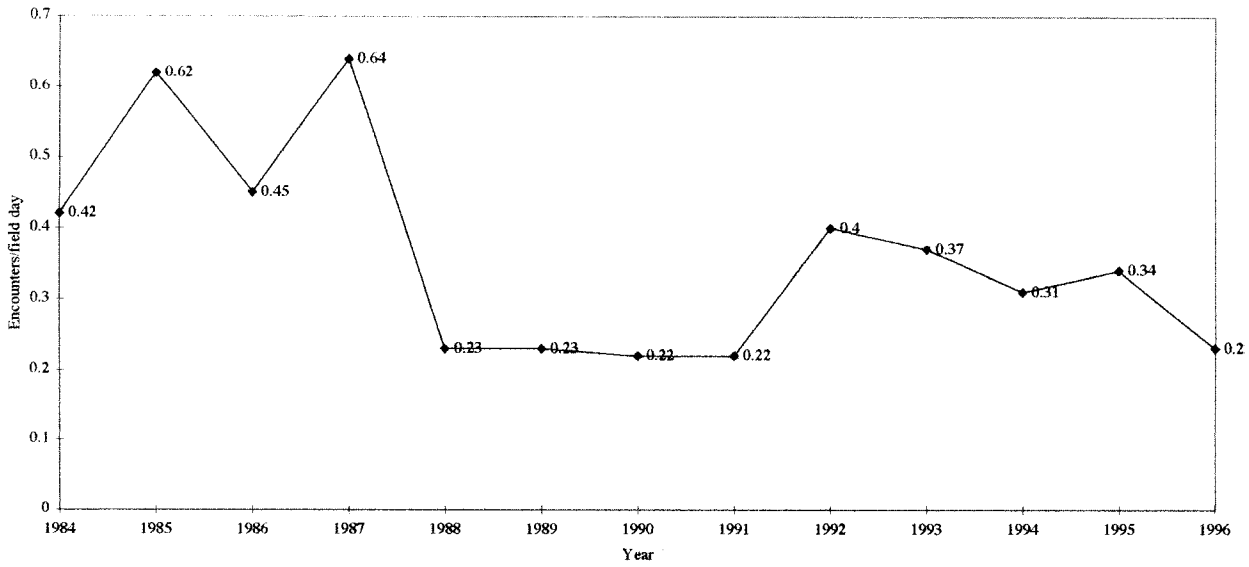


Figure 11. Encounter rates for 2 resident pods by year 1984-1995
 Arcsine transformed ANOVA $p = .60$

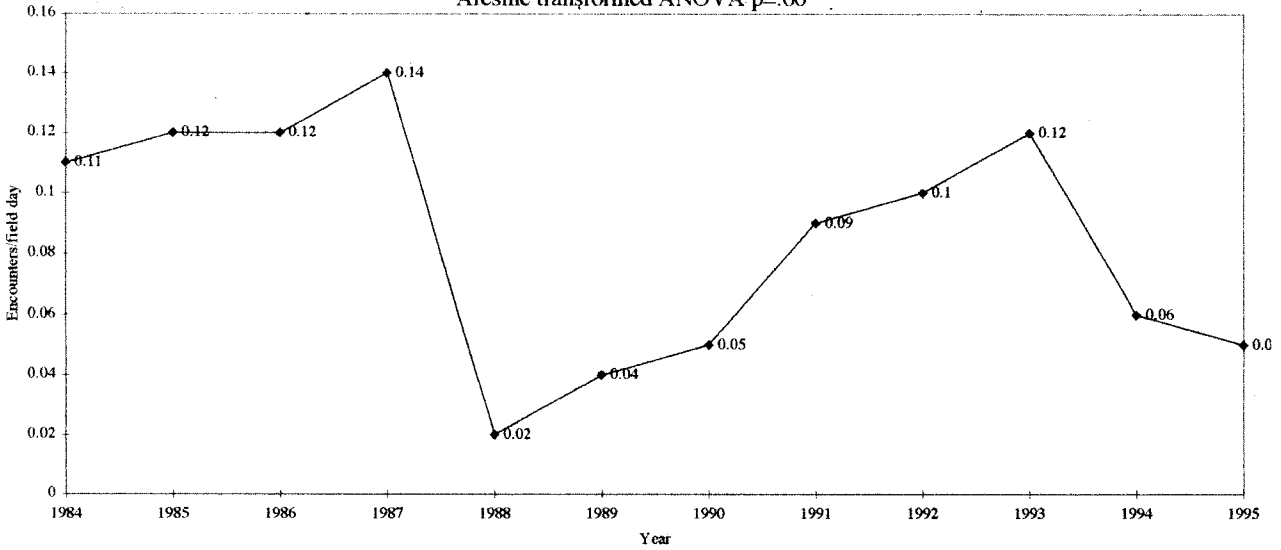


Figure 12. Encounter rate with 3+ resident pods by year 1984-95
 Arcsine transformed ANOVA $p = .055$

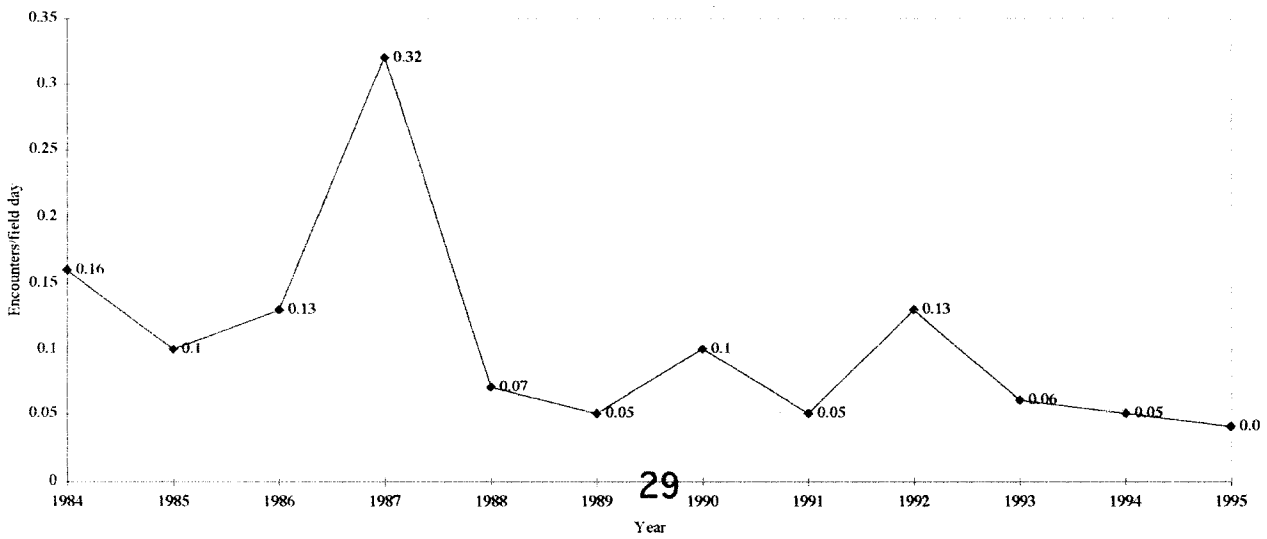


Figure 13. Encounter rates for AT1 transient group by year 1984-96
 Arcsine transformed ANOVA $p=.29$ (84-95)

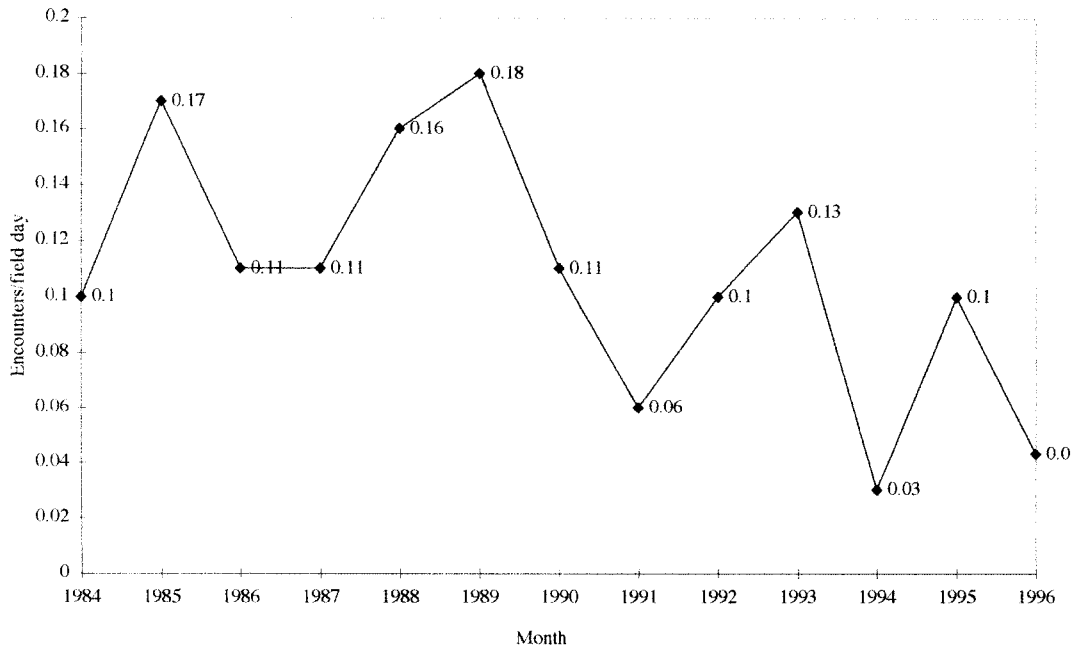


Figure 14. Encounter rate for Gulf of Alaska transients by year 1984-96
 Arcsine transformed ANOVA $p=.50$ (84-95)

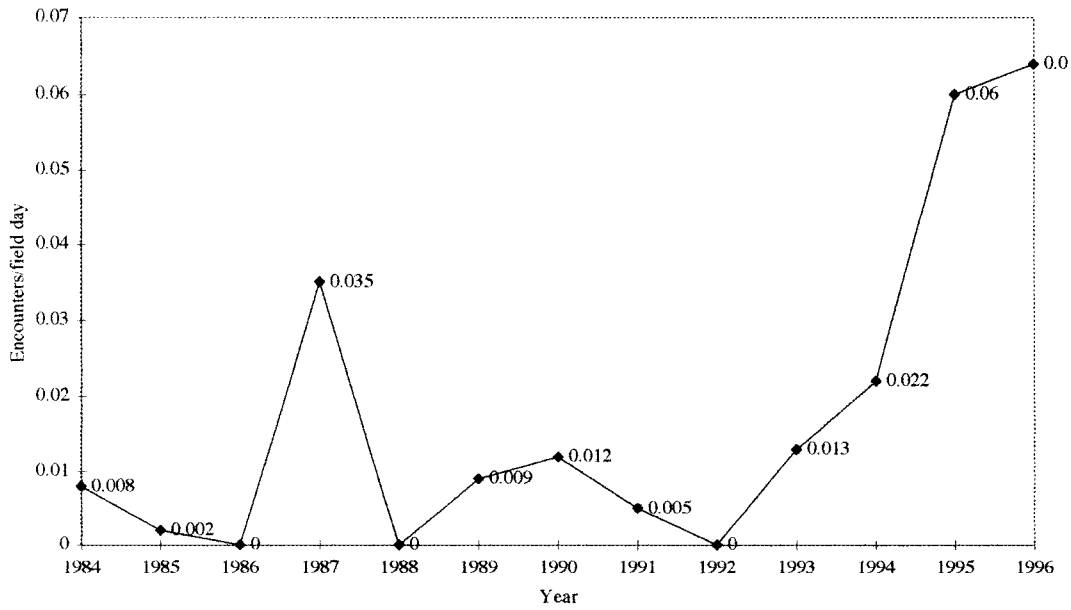


Figure 15. Use of Prince William Sound by resident killer whales by year 1984-1996. (ANOVA $p=0.10$)

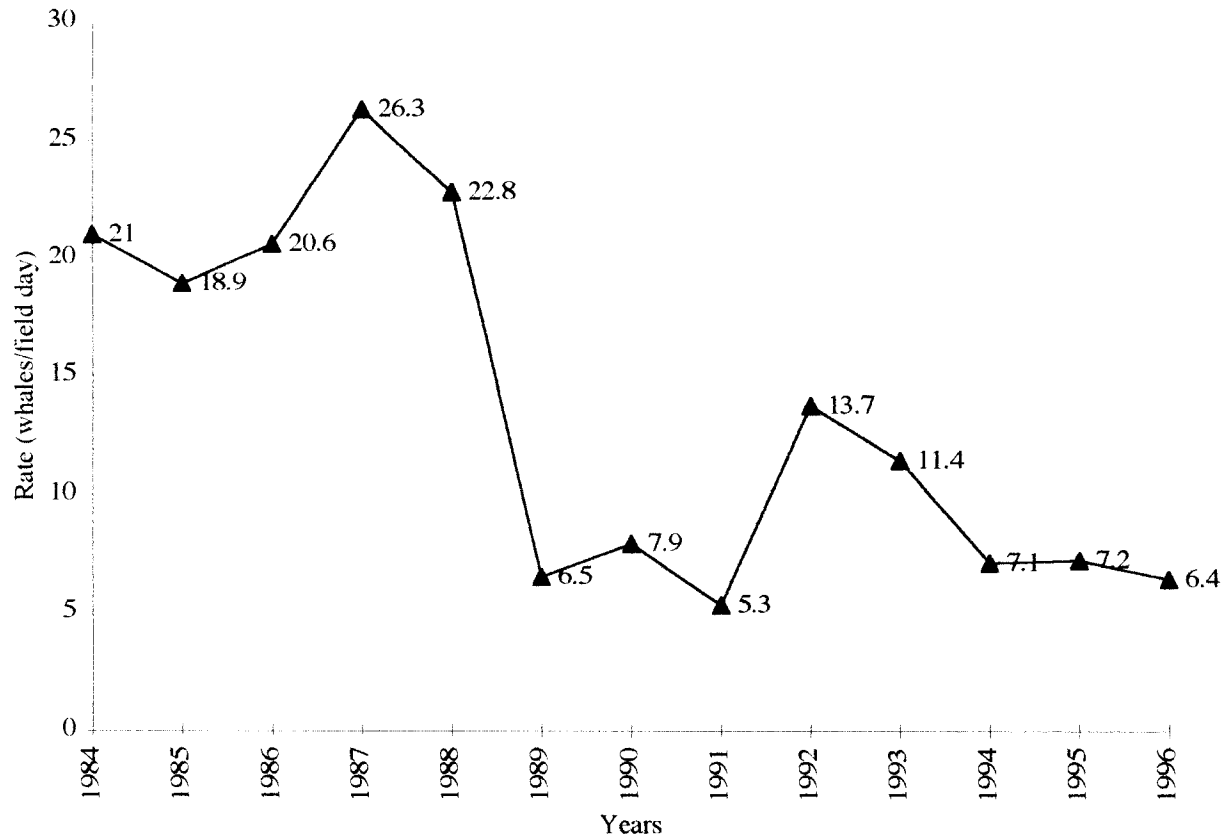


Figure 16. Use of Prince William Sound by resident killer whales by month 1984-1996. (ANOVA $p = 0.002$)

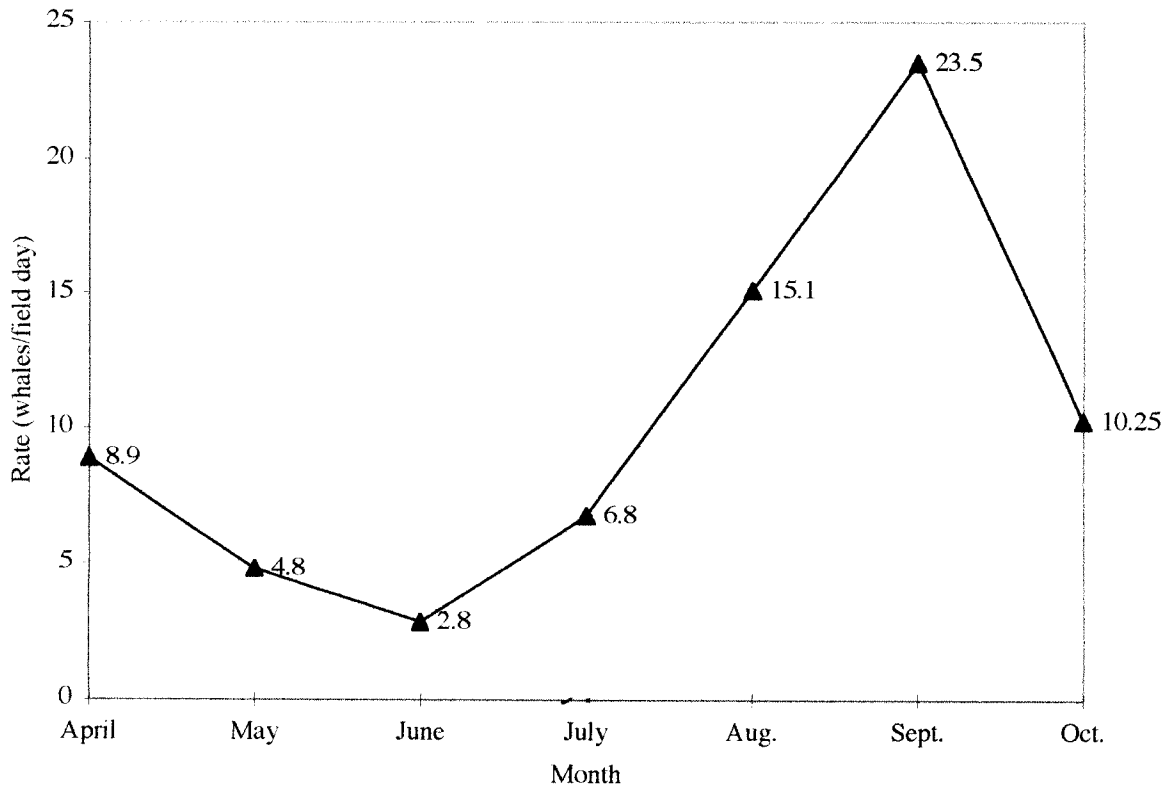


Figure 17. Use of Prince William Sound by transient killer whales by year 1984-1996. (ANOVA $p = .02$)

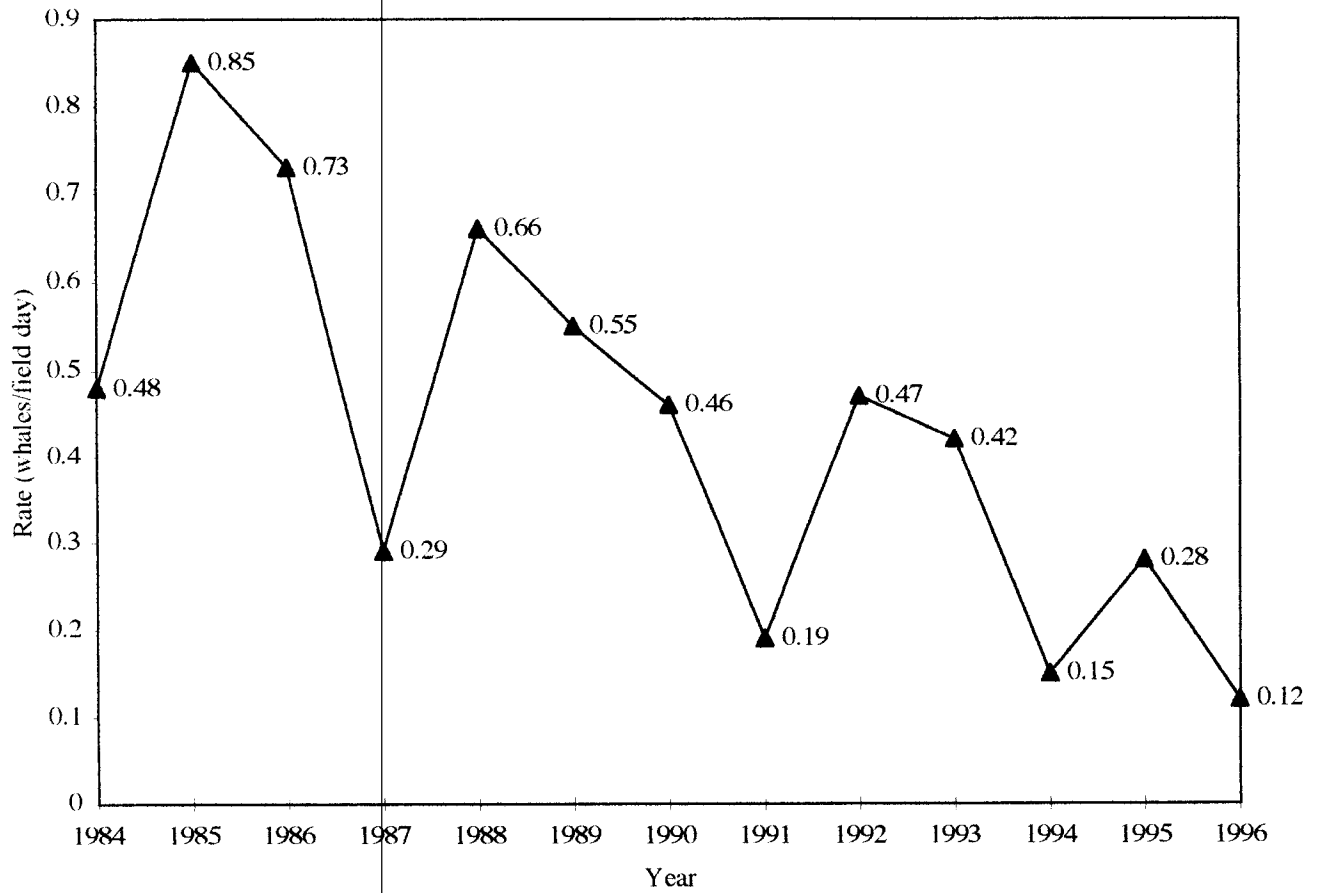
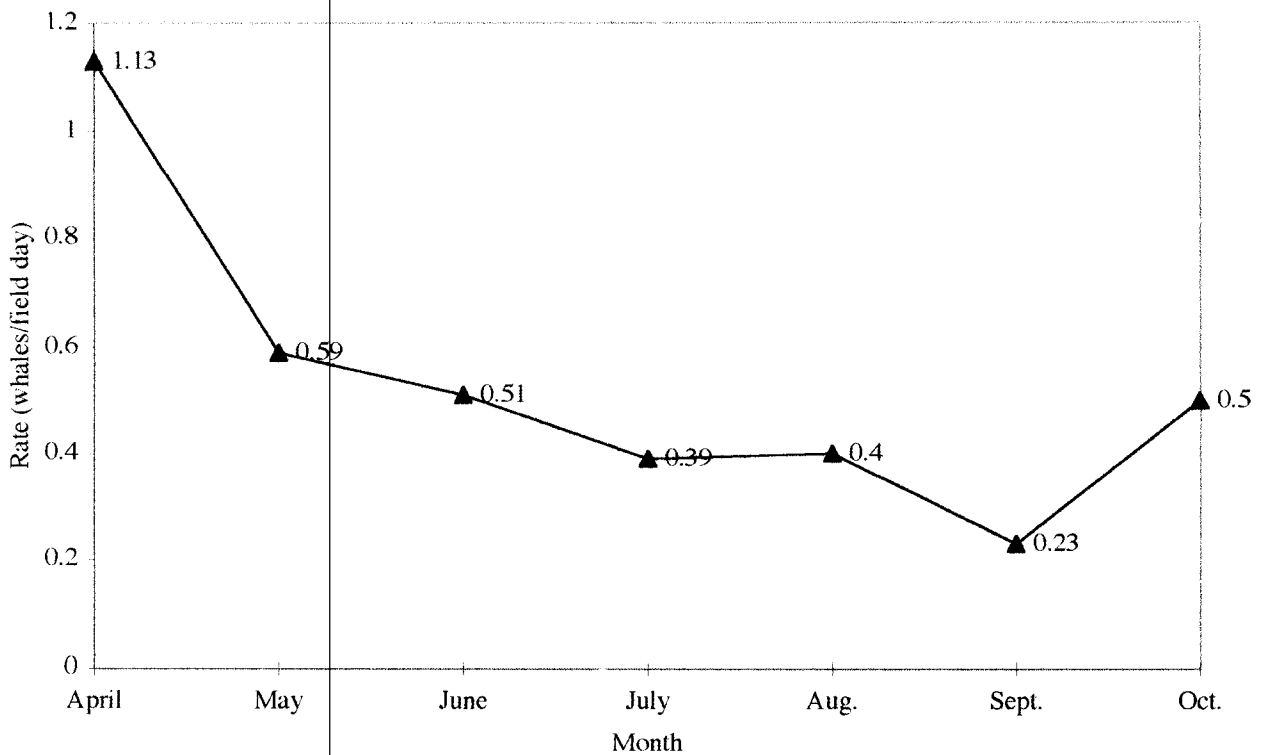


Figure 18. Use of Prince William Sound by transient killer whales by month 1984-1996. (ANOVA $p = .50$)



been a decline in the AT1 group encounter rates since 1989, although it is also irregular and not statistically significant. The higher rate of encounters with AT1 whales in 1988 may be due to directed searching for these whales as part of a focused study of this group (Saulitis, 1994). In 1989 we speculate that the availability of oil injured harbor seals in the southwestern Sound resulted in an increase in AT1 encounters.

Perhaps a more telling measure is the change in use of the area as measured in whale days. Whale days are the actual number of whales (not pods) observed per day of field effort. This analysis indicates a decline in use of the Prince William by both resident whales and AT1 transient whales over the period 1984-1996. For resident whales, there appeared to be a dramatic change at the time of the oil spill, with slight recovery in 1992 and 1993 and then a continuing decline.

Thus, encounter rates (except multi-pod resident encounters) have not declined significantly over the years, but the actual rate of use (measured in whale days) clearly has declined for both resident and transients. This reflects the reduced number of encounters with larger pods that were photographed much more frequently prior to the spill. These include AB pod, AN20 pod, AD5 pod, AD15 pod and AJ pod. The decrease in use is also the result of a decline in the number of encounters with three or more pods (multi-pod encounters). The reduced use of the area by the AT1 transients is undoubtedly due, in part, to a smaller group size per encounter. This is likely a result the reduced number of surviving members in this group. Also, the reduced availability of harbor seal prey may require the whales to forage over greater distances and may also favor a reduced group size. This might also maintain fairly high encounter rates despite the reduced number of whales.

POPULATION GENETICS

Introduction

Observational studies have long indicated that killer whales off the coast of North America do not form a single homogeneous population, but in fact are highly structured. Two feeding specialist types have been identified: the so-called residents, which feed on fish, and the marine mammal-eating transients. By the end of 1996 the genetic separation of residents and transients was well established (Matkin *et al.* 1997b). Furthermore, patterns of genetic differentiation were shown to exist at finer scales. In the 1996 study (Matkin *et al.* 1997b) we reported that the residents of Prince William Sound and vicinity consist of two mitochondrial genotypes, corresponding to the genotypes of the two known populations of resident killer whale found in the nearshore waters of British Columbia. This finding was unexpected because in British Columbia the populations are parapatric and do not associate, whereas in Prince William Sound they are not only sympatric but associate freely (Matkin *et al.* in prep.) We also reported the existence of three genetically-distinct transient groups, two of which are found in Prince William Sound.

In 1997 we examined the population structure of Prince William Sound residents in more detail. In particular, we biopsied more individuals, focusing on pods that were unrepresented or poorly represented in our previous efforts. Our objectives included determining whether any pods were made up of a mixed mitochondrial lineage, and whether the genetic differences between the groups of pods matched behavioral differences. If the present co-existence of whales with two genotypes is the result of the dispersal of one or a few females from one population into the other population, we reasoned that any cultural fingerprint of the dispersers would probably have been lost, whereas clear evidence of cultural differences between the groups with different genotypes would indicate that a movement of substantially intact pods or groups of pods had occurred.

A new approach in 1997 was the use of a polymerase chain reaction-based method to determine the sex of biopsied killer whales. Adult males killer whales can be recognized because their dorsal fins elongate following puberty. However, juveniles are not readily sexed unless their genital area is clearly seen by field researchers, and adult females may be confused with juvenile males. Thus, many individuals are often not reliably sexed until they develop adult male characteristics or appear with a calf.

In a major extension of the genetic analysis of Prince William Sound killer whales, we began microsatellite profiling in 1997. Microsatellites are highly variable nuclear DNA loci that are short enough to amplify readily using the polymerase chain reaction. They are appropriate markers for investigating a wide variety of population properties, including mating systems, inbreeding levels, effective population size, and the extent of population subdivision (Queller *et al.* 1993). The microsatellite analysis, when completed, will also compliment the mitochondrial analysis. Since mitochondria are only inherited maternally they faithfully record long term patterns of female movement. Microsatellites, on the other hand, reflect both male-and-female-mediated patterns of gene flow and will allow us to determine whether intermating between killer whale populations occurs.

Methods

Biopsy Samples

Biopsy samples for DNA and contaminant analysis were collected from free ranging killer whales by NGOS researchers following the method of Barrett-Lennard *et al.* (1996). Collections were made during May through August 1997, in the waters of Prince William Sound, the Kenai Fjords region, and adjacent parts of the Gulf of Alaska. The skin portion of each sample was 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 purified from the samples by protein digestion, phenol-chloroform extraction, and alcohol precipitation using standard protocols.

Mitochondrial DNA Sequencing

The entire mtDNA-D-loop region was sequenced from DNA samples obtained in 1997 using the methods described in Matkin *et al.* (1997b). Because the amplified D-loop fragment was too long (943 base pairs) to be entirely resolved in one direction, we ran two sequencing reactions, one from each end of the fragment. Approximately 400 bp in the centre of each fragment were read from both directions. The sequences were resolved on and read by an Applied Biosystems 377 automated DNA sequencer and checked by eye. The program CLUSTRAL W was used to align the 1997 sequences with killer whale sequences obtained in Prince William Sound in previous years, with sequences obtained in a parallel study in British Columbia, and with sequences from Icelandic whales. Finally, sites found to differ in the alignments were rechecked by eye.

We used a maximum likelihood inference method (reviewed in Swofford *et al.* 1996) to further evaluate patterns and hypotheses concerning historical relationships between killer whale groups that were described in Matkin *et al.* (1997b), and new hypotheses that arose out of the acoustic study described in this report. The inference was performed using the program PHYLIP (Felsenstein 1993). The procedure used was as follows: the sequences were bootstrapped (randomly resampled with replacement) 1000 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 1000 maximum likelihood trees.

DNA-based Sexing

The sexing protocol used was modified from Richard *et al.* (1994), and Palsbøll *et al.* (1992). The method used the polymerase chain reaction (PCR) to amplify a 145 bp region of the SRY region of the Y chromosome. Thus, the presence of amplified DNA of the target size after PCR indicated that a sample of DNA was from a male, no amplification in the same size range occurred with DNA from a female. The SRY primers used were 5'CATTGTGTGGTCTCGTGATC-3' and 5'AGTCTCTGTGCCTCCTCGAA-3'. Two additional primers were also added, as a test of the reaction conditions. The test primers amplified a 727 bp region of the mitochondrial control region. When the reactions were run under the conditions described by Richard *et al.* (1994) and visualized on a 2% agarose gel using UV light and ethidium bromide, the mitochondrial band was generally much brighter than the SRY band. The band strengths were equalized by experimentally reducing the annealing and extension times, to bias the reaction in favour of the shorter sequence. We were able to obtain approximately equal band strengths in these experiments with 35 cycles of 60s at 94°, 44s at 56°, and 30s at 70°.

Microsatellite Analysis

Primers developed for cetacean microsatellite analysis in other studies were tested for their ability to amplify microsatellite loci in killer whales. Non stringent amplification conditions were used initially, including annealing temperatures approximately 10° C lower than the melting temperatures of the primers. The amplification products were visualized on a 2% agarose gel using ethidium bromide and UV radiation. When a given primer set produced an amplification product that was similar in size to that described in the original study, an empirical optimization procedure (based on Innis and Gelfand 1990) was used to improve the selectivity and yield of the reaction. One of each pair of primers was then radioactively end-labelled using the following method: 50 pmol primer, 10 units polynucleotide kinase (PNK), 1X PNK buffer, and 10 μ Ci [γ -³³P]ATP were incubated in a reaction volume of 50 ul for 35 min at 37°, and 5 min at 65°. PCR was then run under the optimized conditions in 10 ul reactions using 1 pmol of labelled primer, 2.5 pmol of the same primer unlabelled, and 6 pmol of the reverse primer. The PCR products were resolved in a 0.4 mm thick 30 X 40 cm denaturing gel made with 5% Long Ranger™ acrylamide solution in 7.0 M urea. The gel was exposed to Kodak BioMax™ film for 12 to 48 hours, and developed using standard methods. Amplified microsatellite products were identified by the presence of stutter bands (Hauge and Litt 1993), and their sizes were determined by reference to a known DNA sequence run on the same gel.

Initially, each primer set was tested on DNA from 40 killer whales that were believed to be unrelated, including resident and transient individuals from both British Columbia and Prince William Sound. Those primer sets that revealed microsatellite polymorphisms in the test group were used to type all individuals in the Prince William Sound populations (work in progress); no further analysis was conducted with primer sets that failed to reveal polymorphisms in the test.

Results

Biopsy Samples

Prior to 1997, DNA was obtained from a total of 54 biopsy dart samples, from five unidentified carcasses, and from one identified carcass. In 1997, 23 new full-sized samples were obtained by biopsy dart. Genomic DNA sufficient for multiple tests (18-148 ug) was successfully extracted from each sample, bringing the total number of DNA samples collected from unique individuals in and near Prince William Sound to 83. One individual from a formerly unsampled

pod (AX pod) was successfully biopsied in 1997, and additional biopsies were obtained from three pods that were poorly represented by samples in the past. AN, AG and AJ were represented by 1, 1, and 2 samples respectively at the end of 1996, and by 8, 2, and 7 samples by the end of 1997.

Mitochondrial DNA Analysis

As in the previous year of the study, a single individual from each set of maternally-related individuals was selected for mitochondrial D-loop sequencing. We also sequenced all individuals for which maternal relationships were not known. All individual whales sequenced are listed by pod in Table 7.

Table 7. Killer whales analyzed for mtDNA D-loop sequences.

Group type	Pod	Individuals sequenced
AT1 transients		AT1, AT9, AT10, AT13 [†] , AT14, AT17 [†] , AT18, AT19 (carcass)
Gulf of Alaska transients		AT64, AC2, AU2, AU3, AU4
Residents	AB	AB3, AB4, AB5, AB14, AB17, AB26
	AD	AD4, AD11
	AE	AE1, AE5, AE10, AE19, AE20
	AG	AG3, AG5 [†]
	AI	AI2, AI3
	AJ	AJ8 [†] , AJ16, AJ17
	AK	AK1, AK8, AK10 [†]
	AN	AN1, AN7 [†] , AN8 [†] , AN12 [†] , AN35 [†] , AN46 [†]
	AS	AS12, AS-female*, AS-male*
	AX	AX 31 [†]
Unknown		5 samples from carcasses

Killer whale and pod names based on Heise *et al.* (1991). Pods are associations of individuals that are stable over many years. Because long term movements of transients between social groups in British Columbia have been observed (G. Ellis, unpubl. data), we have not divided transients into pods.

[†] Whales biopsied in 1997.

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

When the sequences were aligned and compared with each other and with Prince William Sound sequences from previous years, nine variable nucleotide sites were found, comprising one insertion/deletion and seven nucleotide transitions. Eight of these variable sites had been identified previously (Matkin *et al.* 1997b), and one was previously unidentified. When the sequences from previous years were examined, variation at the ninth site was also found to be present. The site was probably missed previously because it resolved poorly in one direction. A careful reexamination of all sequences failed to reveal any additional variable sites, and the new site did not define any new haplotypes. Inclusion of new individuals and a new pod (AX) also did not reveal new haplotypes, and the total number of mtDNA D-loop haplotypes found in killer whales from Prince William Sound and the vicinity remains at four, as reported in Matkin *et al.* (1997b).

In a parallel study in British Columbia four haplotypes have also been found (L. Barrett-Lennard unpublished data), 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 either the British Columbian or Alaskan killer whales. When all seven haplotypes were compared, the total number of variable nucleotide sites increased to 12, comprising one insertion/deletion, 10 transitions, and 1 transversion. These results are summarised in Table 8, and an unrooted maximum likelihood tree based on the consensus of 1000 bootstraps is presented in Figure 9.

Table 8. **Distribution of mtDNA D-loop haplotypes.**

Group	Range	Haplotype [§]	Pods with the haplotype	Number Sequenced [‡]
British Columbia Northern Residents	central Vancouver I. to central SE Alaska	NR	all 16 known pods	28
Prince William Snd. Residents (1)	Prince William Snd. and adjacent waters	NR	AB, AG, AI, AJ, AN, AX	20
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, L	7
Offshores*	pelagic waters from SE Alaska to California	OFF	†	7
British Columbia Transients	east of 142° longitude to California	BCT	†	19
AT1 Transients	Prince William Snd. and adjacent waters	AT1	†	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

Pod names from Heise *et al.* (1991) and Ford *et al.* (1994).

[§] 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.

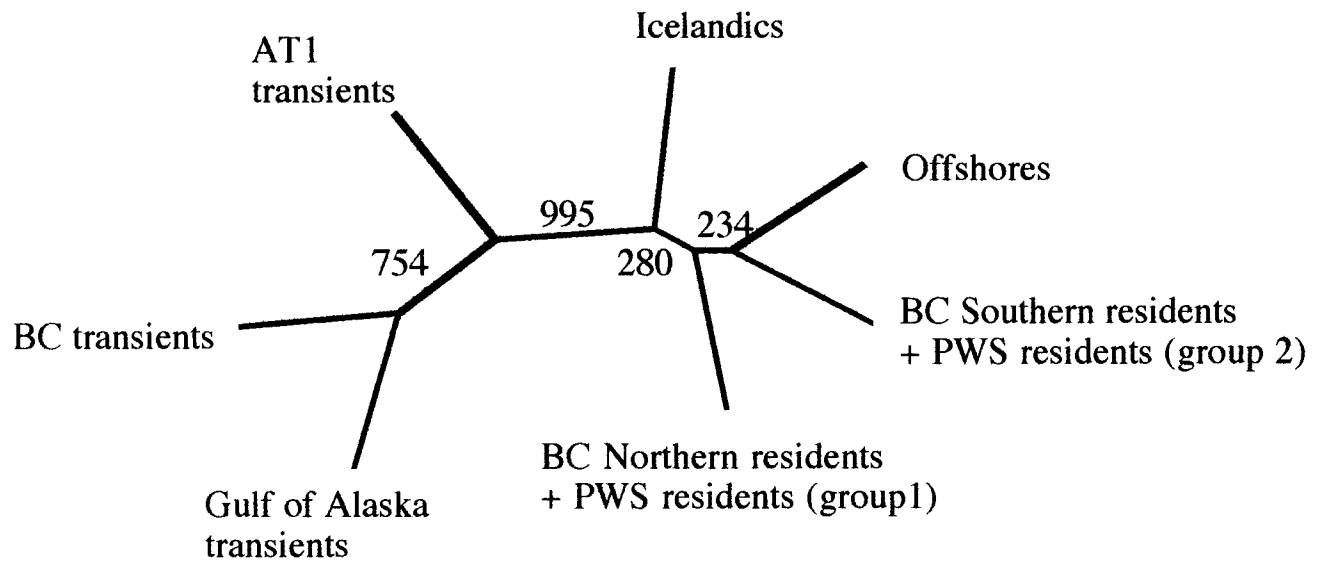


Figure 19. Consensus of 1000 bootstrapped maximum likelihood trees. The numbers indicate the number of bootstraps which had the same combinations of populations to the left and right as shown in the consensus. For example, the Gulf of Alaska transient and the British Columbia transient groups shared an ancestor more recently with each other than with any other group in 754 out of 1000 hypothetical trees, and those groups along with the AT1 transients shared an ancestor more recently with each other than with any other group in 995 of 1000 trees.

Genetic Sex Determination

A total of 22 whales were genetically sexed by PCR amplification of the SRY region. Control DNA from whales of known sex used as a blind test during each amplification run produced correct determinations in all cases. An example of a sexing run is shown in Figure 20. The non-sex specific mitochondrial fragment which was amplified simultaneously with the SRY region as a test of reaction conditions did not appear in two of the reactions, but amplified according to expectations when the reactions were repeated. The results of the sexing analyses are presented in Table 9.

Table 9. Sexes of individual whales determined by SRY region amplification.

Individual	Sex	Individual	Sex
AB27	female	AK12	male
AB45	male	AK13	female
AC2	female	AN12	female
AD4	female	AN46	male
AE15	male	AS?*	female
AE17	female	AT10	male
AE18	male	AT64	female
AE19	male	AU2	female
AE20	female	AU4	male
AK8	male	AX31	male
AK10	female		

Killer whale names based on Heise *et al.* (1991).

* believed to be from AS pod and female-like in appearance, but not individually identified.

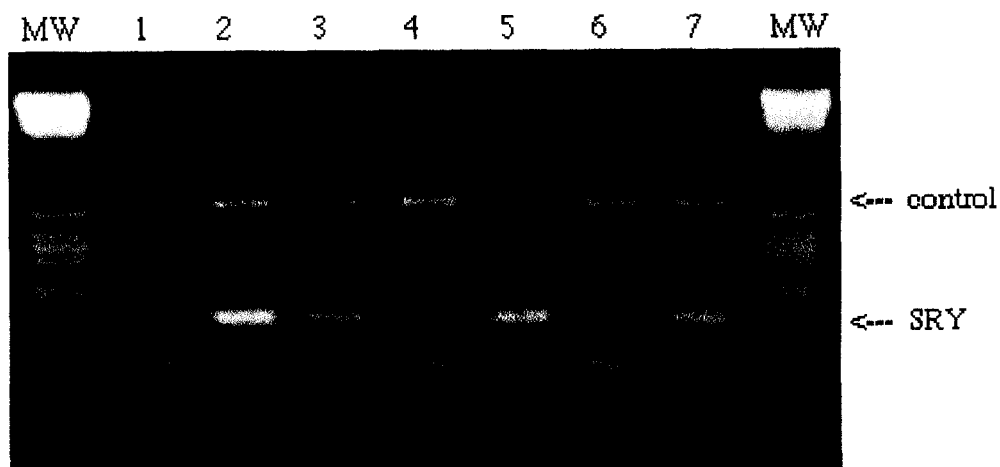


Figure 20. Photograph of ethidium bromide-stained agarose gel showing PCR amplification of both the SRY region of Y chromosome and a fragment of mitochondrial DNA as a control. The control band is a 727 nucleotide region of mitochondrial DNA, and the SRY region is approximately 145 base pairs. Lanes labelled MW contain a molecular weight marker. Lanes 1 and 2 contain DNA from known female and male whales respectively. The remaining lanes contain DNA from whales that have not been sexed by field observations. The presence of SRY bands in lanes 3, 5, and 7 indicate DNA from males; the absence of SRY bands in lanes 4 and 6 indicates female DNA.

Microsatellite Analysis

We have tested total of 12 cetacean microsatellite primers sets (from Buchanan *et al.* 1996, Schlötterer *et al.* 1991, and Valsecchi and Amos, 1996) on Prince William Sound killer whales to date. Of these, three failed to amplify and three were monomorphic. We are presently typing all Prince William Sound killer whales at the remaining six loci, which amplify well. Three to eight alleles have been identified at these loci thus far. Figure 21 is a radiograph showing microsatellite variation in resident killer whales.

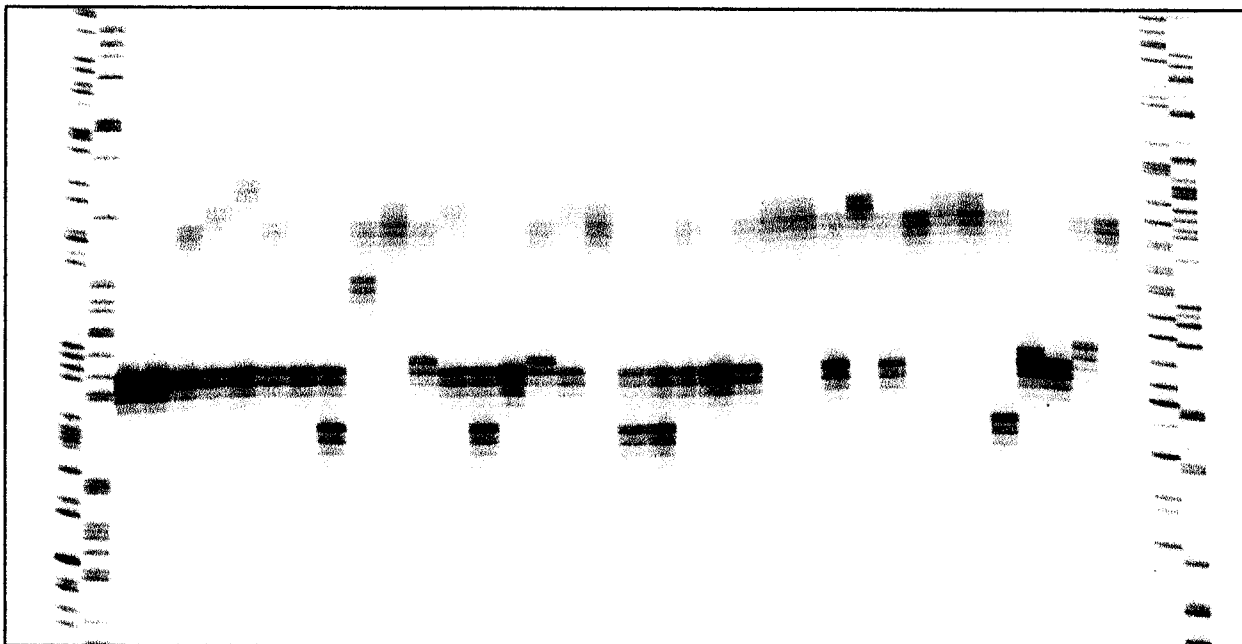


Figure 21. Acrylamide gel radiograph of ^{33}P -end-labelled PCR products of a microsatellite locus amplified from resident killer whale DNA. The first two and last two lanes are from a sequencing reaction used as a size reference; between them are lanes containing alleles of 35 biopsy-sampled resident killer whales. The third lane from the right is a negative control.

Discussion

Population Structure of Prince William Sound Killer Whales

The mitochondrial DNA analysis in 1997 extended earlier findings (Matkin *et al.* 1997b) of fixed mtDNA differences between populations. While the differences between resident and transient killer whales are consistent with findings from British Columbia (Barrett-Lennard, unpubl. data), the genetic divisions within each type are unique. Here, we treat each type separately.

Resident killer whales

In British Columbia, three groups of residents have been distinguished genetically: the so-called offshores, southern residents, and northern residents. These three groups had previously been distinguished based on their distribution, acoustic repertoires, and association patterns (Ford *et al.* 1994). Their ranges occasionally overlap, but they are normally sighted in different regions of the coast and have never been seen to associate. Each is monomorphic for a given mtDNA D-loop haplotype (unpubl. data). Because the absolute genetic differences between each group are minor (c. 1% divergence), they would most parsimoniously be considered maternal lineages within a single population if there were not independent evidence of their functional separation.

Prince William Sound resident killer whales differ from those just described in that the two genetic types associate and are sympatric (see Matkin 1994). Furthermore, one type (referred to here as *PWS group 1*) is identical in mtDNA D-loop haplotype to the B.C. northern residents, and the second (*PWS group 2*) is identical to the B.C. southern residents. Several sequences of events could explain these patterns. For example, an ancestral resident population may have divided into two parapatric subgroups, which diverged genetically and now make up the southern and northern resident communities. Subsequently, one or more females or pods from the southern resident community moved into the northwest end of the range of the northern residents, and the two groups now overlap in Prince William Sound. Alternatively, pods from a single group with two haplotype lineages may have colonized the entire coast, and the present pattern of haplotype distributions arose by chance. There is little possibility of going beyond this type of speculation on the basis of the mtDNA haplotype distributions alone. However, the discovery that the pods of Prince William Sound killer whales fall into two discrete groups based on vocalizations (this report), and the fact that the acoustic divisions are perfectly congruent with the genetic divisions, provides valuable insight. For example, because both groups have different acoustic traditions, it is unlikely that the present situation in Prince William Sound arose by the random colonization of the coast by individuals with differing haplotypes but a common tradition. Similarly, it seems unlikely that the two vocal traditions would have been preserved if only one or several females of one type emigrated into pods of the other type. Rather, since the call traditions apparently survived a joining event, we believe it more likely that substantially intact pods moved into proximity, creating the present situation of sympatric coexistence of distinct groups.

Transient killer whales

In British Columbia, all transients analyzed to date have a common mitochondrial D-loop haplotype. This haplotype has not been found in Prince William Sound, however, the haplotypes of both the AT1 transients and Gulf of Alaska transients are closely related to it. The genetic differentiation of the three transient groups follows patterns already established in observational studies. It has been noted that although individual transients are capable of long-range movements (Goley and Straley 1994), none have been sighted on both sides of a line east of Prince William Sound (at approximately 142°W longitude; Barrett-Lennard *et al.* 1995). This division is consistent with the genetic differences between B.C. and Gulf of Alaska transients. The AT1 transients are only commonly seen in and near Prince William Sound. They differ acoustically and behaviourally from sympatric Gulf of Alaska transients, and do not associate with them (Saulitis 1993). The results of the maximum likelihood analysis are consistent with a long period of genetic separation of transients from residents, and significant but lesser separation between the three transients groups.

Genetic Sex Determination

Knowledge of sex ratios at all age classes is required for the construction of accurate life tables, and thus is an important component of population dynamics studies. Similarly, in field studies of social behaviour it is extremely helpful to know the sexes of focal individuals. In this paper we

have shown the SRY amplification method to be a simple and accurate method of molecular sex determination, which can be readily applied in cetacean studies involving biopsy sampling.

Microsatellite Analysis

The microsatellite analysis described in this paper is still underway, and preliminary results are not presented here. However, we have demonstrated that sufficient microsatellite polymorphism exists to investigate general patterns of gene flow, and describe population sub-structuring. We anticipate reporting the results of this analysis in our 1998 report.

ENVIRONMENTAL CONTAMINANTS

Introduction

Calambokidis *et al.* (1990) found concentrations of total PCBs of greater than 100,000 ppb and total DDT levels greater than 400,000 ppb in some samples from whales stranded in Washington State, British Columbia and Alaska between 1976 and 1989. However, in this study the sample size was small and the levels were extremely variable between individuals. At the time there was no obvious explanation for this variability. With the lack of reproduction in the AT1 transient group, we became concerned that high contaminant levels might be a factor that would impede recovery of the group from its sharp decline at the time of the EVOS. Development of techniques for the biopsy of free-ranging killer whales (Barrett-Lennard *et al.* 1996) provided the opportunity to obtain blubber tissue for contaminant analysis. Since this phase of the project was initiated in 1994, a total of 60 killer whale blubber samples that were suitable for environmental contaminant analysis have been obtained of individually identifiable whales; three were duplicate samples, ten were obtained from transient whales (one duplicate) and 50 from resident whales (two duplicates).

Analytical Methods

Killer whale blubber samples were analyzed for selected chlorinated hydrocarbons (e.g., dioxin-like CBs, DDTs) using rapid high performance 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-dioxin; 250ng) were homogenized, decanted 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 chlorinated 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.

All analytical results were corrected for percent lipids in the sample before comparisons were made. Lipid percentage varied depending on the location on the whale that the sample was taken and contaminant levels have been found to be directly proportional to the percentage of lipids in the tissue

sample. Analytical results, total PCBs and total DDTs, for individuals were placed in several sample groups to examine differences in levels of contaminants based on population, sex, reproductive history, genealogy and pod. Groupings included all resident whales, all transient whales (includes AT1 group and GOA transients), resident reproductive females, resident males, first born resident whales, non-first born resident whales, AB pod whales and AK pod whales. Descriptive statistics including sample mean and standard deviation and confidence intervals ($p=.05$) for the population mean were developed. Comparisons between the means of selected pairs of these groupings were made using a student's t-test for populations with unequal variance.

Results

The levels of polychlorinated biphenyl (PCB) congeners and DDT and its metabolites showed a wide range among individual samples. However, duplicate samples taken from the same individuals at different times during the 1997 season were extremely consistent once corrections were made for percent lipids in the samples. The PCB congeners 101, 118, 138, and 153 were the congeners that demonstrated the highest levels in all samples. The greatest component of total DDTs was ppDDE in all samples. Transients had total PCB levels over 14 times greater than residents and DDT levels over 22 times greater than residents (Tables 10,11). Residents sample groups showed a wide range in mean levels of total PCBs and total DDTs (Tables 10,11).

Table 10. Levels of total PCBs in selected groupings of killer whales.

	Resident Repro. Females	Resident Males	Resident First Born	Resident Non-First Born	Resident AB pod	Resident AK pod	All Residents	All Transients
n =	16	22	19	17	7	5	52	9
Mean	3349	17,027	25,979	11,618	8814	22,800	14,321	208,111
Stan. Dev.	1680	12,577	14,933	6190	2384	7014	7559	104,736
C.I. (0.05)	823	5255	6715	2942	1766	6148	2851	68,426

Figure 22. Mean levels of PCBs and DDTs in resident killer whales

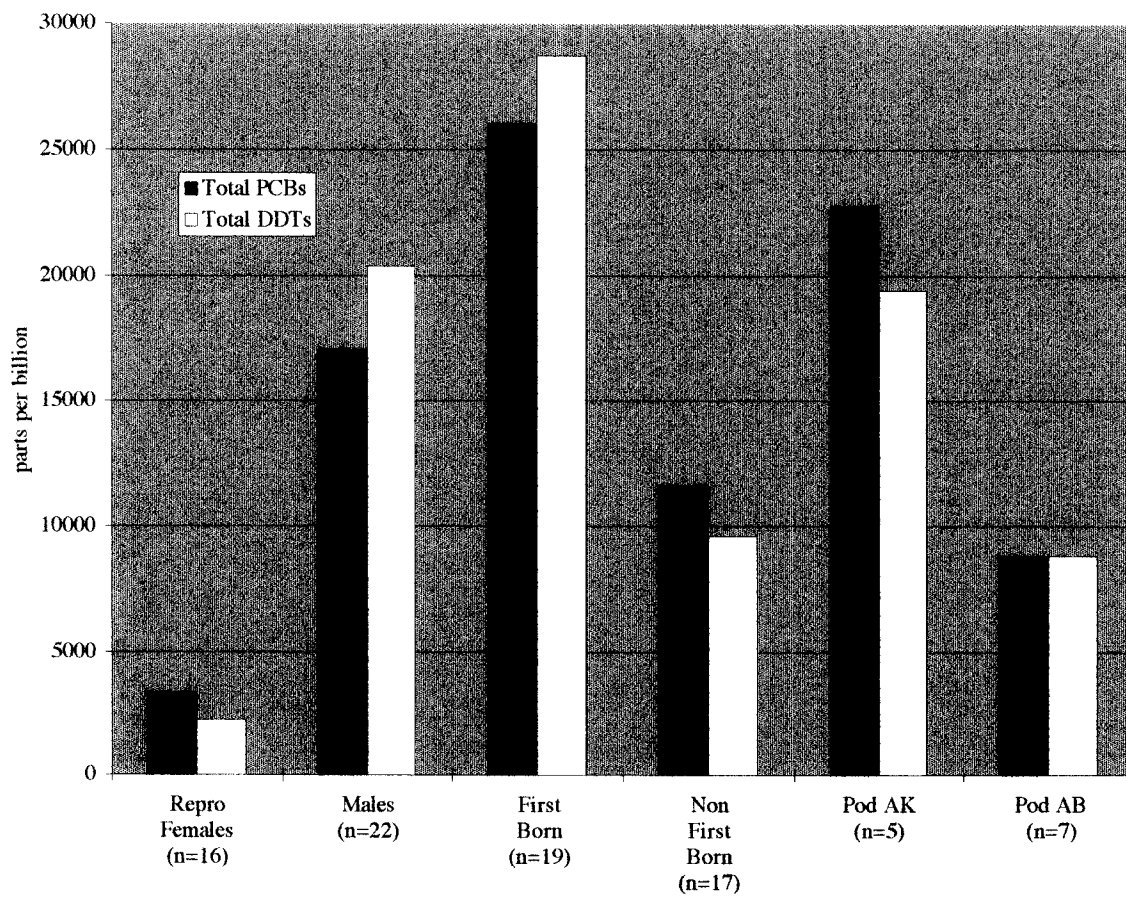


Table 11. Levels of total DDTs in selected groupings of killer whales.

	Resident Repro. Females	Resident Males	Resident First Born	Resident Non-First Born	Resident AB pod	Resident AK pod	All Residents	All Transients
n =	16	22	19	17	7	5	52	9
Mean	2284	20,372	28,737	9594	8843	19,400	14,339	317,777
Stan. Dev.	1707	21,741	22,223	4633	3443	8792	7367	218,562
C.I. (0.05)	836	9085	9992	2202	2551	7063	2636	142,791

The comparisons between sample groups demonstrated a highly significant difference ($p \geq .01$) between sample means in all pair-wise comparisons except comparison of pod (Table 12). For pod AK and pod AB where there was a significant difference in mean total PCB levels and a marginally significant difference in mean total DDT levels.

Table 12. Level of significance for student t-tests comparing total PCB and total DDT levels in paired sample groups with unequal variance. (p values).

Sample Means Compared	Total PCBs	Total DDTs
All Residents--All Transients	.0005**	.003**
Resident Reproductive Females-- Resident Males	.00005**	.00002**
Resident Reproductive Females-- Resident First Born Whales	.000007**	.00009**
Resident Reproductive Females-- Resident non-First Born Whales	.00002**	.00002**
Resident First Born Whales-- Resident non-First Born Whales	.001**	.002**
Resident pod AK--Resident pod AB	.01*	.06^

** highly significant

* significant

^ marginally significant

Discussion

A larger sample size has indicated an even wider difference in the levels of PCB congeners and DDT and its metabolites than indicated initially in resident versus transient whales. The difference is highly statistically significant. This underscores the effect of difference in diet on contaminant levels in the fish eating resident whales and marine mammal eating transient whales. The variability in contaminant levels found by Calambokidis *et al.* in stranded killer whales is apparently due to samples from different populations (resident and transient) that had different diets. The high levels of PCBs and DDTs that he found are directly comparable to levels we have found in known transient whales. Although there is no unequivocal evidence that high levels of contaminants result in reproductive failure in cetaceans (Addison 1989), we are concerned that there is a linkage between the apparent low rate of reproduction in transients (zero in the AT1 group) and high contaminant levels. Contaminant amounts we have found are comparable to those found to cause reproductive problems in other marine mammal species (Helle *et al.*, 1976)

The contaminants that are present in the Gulf of Alaska and assimilated by the killer whales likely travel in weather systems from southeast Asia or China where DDT and PCBs are still in wide use. The materials may volatilize in these warmer regions, move northward in weather systems and condense and fall with rain in cooler northern regions (Iwata *et al.* 1993, Iwata *et al.* 1994). The contaminants are not excreted but bioaccumulate in the fatty lipids as they move up the food chain.

The highly significant lower contaminant levels in reproductive females versus males supports the hypothesis that mothers pass the majority of their stored contaminants to their offspring via lactation. The highly significant lower contaminant levels in second born offspring than in first born offspring are probably due to the first born receiving the contaminants accumulated in the mother's lifetime prior to first reproduction (average 15 years) and contaminants she may have received from her mother. It is clear that factors such as age, sex, reproductive status and other life history parameters have a large influence on the contaminant levels observed in a particular individual.

Although the samples from pod AB and pod AK contained whales that displayed a similar range of sex and life history attributes, they had very different in contaminant levels. Additional samples are required to confirm this apparent difference, however, we suspect it may be due to pod-specific feeding preferences. AK pod frequently travels nearshore and has been observed feeding on halibut as well as salmon, while AB pod has not. Conceivably, AK pod may feed more extensively on long-lived bottom fish or other fishes with higher contaminant levels.

ACOUSTIC ANALYSIS

Introduction

At least three different killer whale populations use Prince William Sound/Kenai Fjords region, the resident killer whale population, the AT1 transient population, and the Gulf of Alaska transient population (Heise *et al.* 1991; Leatherwood *et al.* 1984a, 1984b, 1990; Matkin & Saulitis 1994). Although the region is undoubtedly important for foraging (Matkin *et al.* 1997a) members of the most prominent population, the resident killer whale population, also uses the region for 'social gatherings'. Such gatherings are reported for resident killer whales in other areas (Bigg *et al.* 1990, Ford *et al.* 1994), and probably function as an opportunity for mating and maintenance of population cohesion.

Thus far, vocal dialects within killer whale populations have been described only in resident killer whales. They are probably of great importance during multi-pod gatherings because they allow individuals to distinguish between relatives and non-relatives. Dialects appear strongly correlated with the social organization of resident killer whales and less with the geographic distances between

their groupings (Ford 1991). The social organization is characterized by lack of dispersal of animals from groups (pods). All offspring stay in their natal group for life and male offspring maintain a very close relationship to their mothers (Bigg *et al.* 1990, Olesiuk *et al.* 1990).

This social uniqueness is probably responsible for the pod-specific dialects which maintain their integrity even though the groups continuously mix and associate with each other (Ford 1984, 1989, 1991; Ford *et al.* 1994a, Strager 1995). Because each member of a pod produces the whole pod specific repertoire of call types, movements of pods can be monitored acoustically. Since their dialects are very consistent over time, relationships between pods can be explained on the basis of similarities of call types. In addition, call dialects can be used to acoustically track particular pods without actually observing the animals. In this study the distinct repertoires of resident killer whales from the Prince William Sound/Kenai Fjords region will be tested for dialect. Conclusions regarding the relationship of pods will be drawn on the basis of acoustical similarities of call types.

Methods

The procedure used to analyze the vocalizations has been developed by Ford (1984) and applied to vocalizations of resident-type killer whales in Norway (Strager 1995), and to an isolated transient group of killer whales called AT1 in Prince William Sound, Alaska (Saulitis 1993).

Selection of recordings

Recordings collected from 1984 to 1994, prior to the initiation of this study. Recordings collected from 1995-1998 will be included in the final report of this study. Only recordings of calls that could be attributed unequivocally to a particular pod were examined in this phase of the study. Because of the variety of recordings from many different observers and the resulting inconsistency in behavioral descriptions, it was impossible to consider only calls from similar behavioral contexts. In order to avoid the complication of situation-related variation in call usage (Ford 1989), the tolerance thresholds for identification of variations of types was set higher than in those of Ford's analysis.

All recordings meeting the above criteria were used to describe the typical call repertoire of a pod. Also, representative samples of each call type were drawn from these tapes for quantitative structural analysis.

The number of accounts when a particular pod was recorded alone varied considerably. For example, AI-Pod was almost always in the company of at least one other pod until 1990 when these whales were recorded alone for the first time. Some pods have been recorded more often than others however, most call types in each pod's repertoire should be identified and their relative frequencies of use correctly determined with additional field work. In Table 1 the number of existing recordings and the number of single pod recordings that have been analyzed is displayed for the years examined thus far.

Table 13. Encounters with recordings of six pods in each year of the study period 1984 to 1994.

Year/Pod	AB	AI	AN	AE	AK	AD	Total
1984	36 (8)	25 (0)	17 (1)	16 (3)	7 (2)	12 (2)	113 (16)
1985	13 (3)	6 (0)	8 (4)	11 (4)	2 (1)	2 (0)	42 (12)
1986	6 (0)	2 (0)	5 (0)	1 (1)	1 (0)	1 (0)	16 (1)
1987	7 (0)	7 (0)	8 (0)	0 (0)	0 (0)	0 (0)	22 (0)
1988	4 (0)	0 (0)	3 (0)	0 (0)	2 (1)	0 (0)	9 (1)
1989	4 (2)	1 (0)	1 (0)	1 (0)	2 (2)	0 (0)	9 (4)
1990	19 (1)	11 (3)	19 (2)	10 (2)	6 (2)	3 (0)	68 (10)
1991	3 (0)	8 (2)	6 (2)	10 (3)	10 (4)	5 (1)	42 (12)
1992	21 (2)	12 (2)	17 (1)	10 (2)	5 (1)	1 (0)	66 (8)
1993	1 (0)	1 (0)	0 (0)	3 (1)	0 (0)	1 (1)	6 (2)
1994	3 (0)	3 (1)	2 (0)	6 (0)	5 (0)	1 (0)	20 (1)
Total	117 (16)	76 (8)	86 (10)	68 (16)	40 (13)	26 (4)	413 (67)

The numbers in brackets represent the number of single encounters that have been analyzed to this point. Actual recording durations differed among encounters, so did the vocal activity of the whales.

Data analysis

Qualitative analysis

Most calls of resident killer whales could be classified by ear into discrete categories. The categories were based on distinctive structural characteristics of the calls frequency vs. time contours. A Kay Elemetrics spectrum analyzer equipped with a DSP board that allowed real time signal analysis was used to reveal those characteristics. Samples of each classified category were further analyzed using a computer based bioacoustics workstation called Canary, (version 1.2.1), which ran on a Macintosh platform and was developed by the Bioacoustics Research Program of the Cornell Laboratory of Ornithology.

Distinct call types were named alphanumerically using AKS as an abbreviation for calls from southern Alaska. Numbers were assigned to call types according to the order in which they were identified. There is no hierarchical structure within the numbering system. The appendices i, ii, iii etc. used in combination with some call types indicate the existence of stable variations of the same call type. Call variations appeared as alternate forms of the same distinct call type through shift of particular acoustic variables, e.g. AKS1i can be modified into AKS1ii without the existence of distinct contourbreaks (Fig 23).

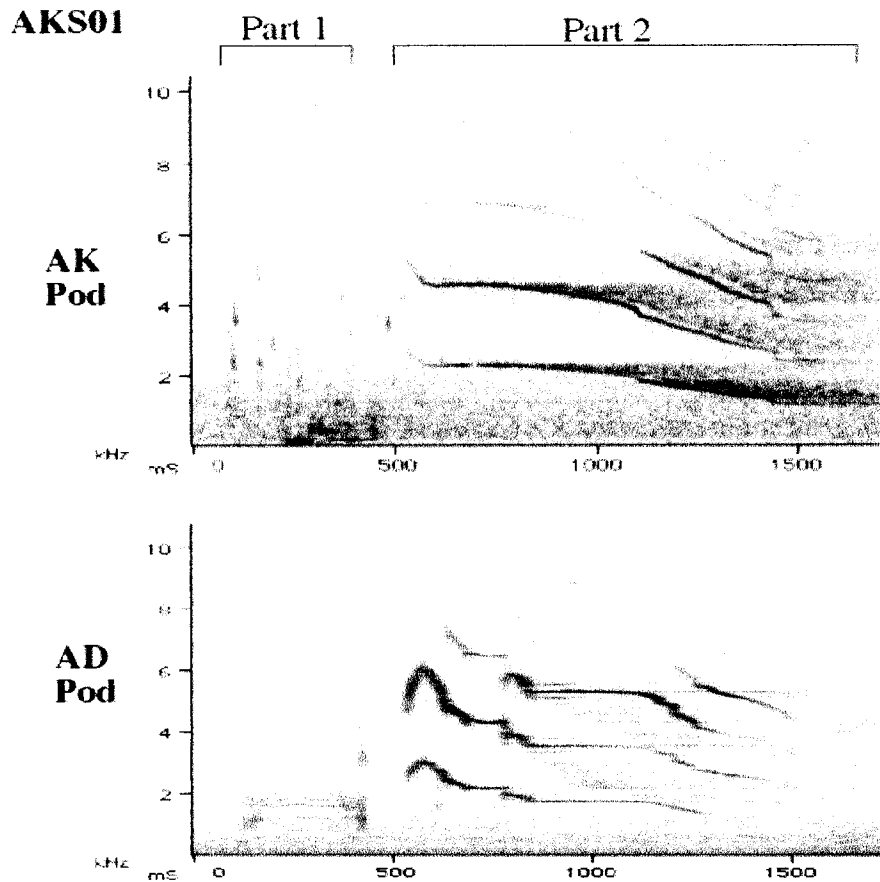


Figure 23. Two call variants of type AKS 01 produced by AK pod (upper picture) and AD pod (lower picture). The call type was produced with or without the initial part through varying duration of part 2.

Quantitative Analysis

Pods which are sharing a great number of call types also can be distinguished from each other if they differ in the frequency of call usage. To determine differences in call usage between pods we used the following call type frequency index:

$$\text{Call-type-frequency Index} = \frac{k}{t^2} * \sum_{i=1}^t c_i$$

- ci: frequency of call types per recording session
- k: number of sessions when call type was recorded
- t: number of recording sessions

Acoustic recordings made from a fixed hydrophone in Prince William Sound during the winter months were compared to the call types from the dialect catalogue of resident pods of this area. The dialect catalogue was developed on the basis of recordings from single pod encounters. These comparisons were used to identify pods present during the winter months.

Vocalizations of AT1 transients and Gulf of Alaska transient types were not a focal part of this study because most of them have been already described and catalogued previously (Saulitis 1993). Their vocalizations are easily distinguished acoustically from residents, because they do not share call types with any resident group and their call types carry unique tonal qualities. The catalogue of AT1 calls will be used to determine the presence of these whales in recordings from the remote hydrophone. Few recordings exist from the Gulf of Alaska transient population. Recordings obtained from these whales will be filed and possibly analyzed at a later time.

The focus of this first year of acoustic analysis was assessing the repertoires of resident killer whale pods that are most frequently observed in the region. Sample recordings from encounters with six pods (AB, AD, AE, AI, AK, AN) which were regularly recorded in Prince William Sound during the years 1984 to 1994 were analyzed. From these recordings 8456 calls were digitized and spectrographically compared. Several other pods will be examined in future analysis if sufficient recordings are obtained. These include AF and AG pod which are occasionally seen in our study area, but are seen more regularly in Southeastern Alaska, and AJ, AS, and AX pods which visit our study area at irregular intervals.

Results

Vocalizations of resident killer whales in Prince William Sound/Kenai Fjords are structurally similar to resident whales from British Columbia and Washington State. They consist primarily of broad band tonal pulse sounds (low frequency component) with intermixed pure tone components (high frequency component) that have a whistle-like appearance on the spectrograph. The sound frequency of these calls ranges from a few hundred hertz to around 11-12 kHz. They have a slightly higher average upper frequency limit than calls from residents in British Columbia and Washington State. However, this difference was not significant.

A total of 28 call types could be identified in the vocalizations of the six pods, AB, AE, AI, AK, AD and AN pod. These call types fell into 18 distinct type categories. Eight of these 18 distinct types showed more than one variation of form. One of these eight had four variation forms, another one three, and the rest had two variation forms. Two examples of variations of the same distinct call type are displayed in Figure 23.

The mean number of call types for each pod was 9.5. It ranged from seven types in AE and AK pod to 15 types in AB pod. Table 14 lists the call types used by each pod. Vocalizations were recorded during a wide range of behavioral categories, such as travelling (slow and fast), feeding, resting, and socializing. Call type and frequency appeared independent of behavior mode with the exception of 'resting' in which the whales did not vocalize or used particular vocalizations more than others and 'slow travelling' when the whales were also mainly silent.

Table 14. List of all identified call types and variation forms in alphanumerical order.

	AB-POD	AI-POD	AN-POD	AD-POD	AE-POD	AK-POD
AKS 01 i				X		X
ii				X		X
AKS 02 i					X	
ii					X	
iii					X	
AKS 03				X	X	X
AKS 04				X	X	X
AKS 05				X	X	X
AKS 06						X
AKS 07	X	X	X			
AKS 08 i	X		X			
ii	X					
AKS 09 i				X	X	X
ii				X		
AKS 10 i	X		X			
ii	X	X				
AKS 11 i	X	X	X			
ii	X	X				
AKS 13	X	X	X			
AKS 14	X	X				
AKS 15	X	X	X			
AKS 17 i	X	X				
ii	X	X				
iii	X	X	X			
iv	X	X	X			
AKS 21				X		
AKS 22	X	X				
Total:	15	12	8	8	7	7

Call type is attributed to pod by an X in the appropriate column. Pods which share call types are grouped together.

From Table 14 it is apparent that pods separate into two clusters based on shared call types. The pods AI, AB, and AN pods share call types and pods AD, AE, and AK share call types. There was no apparent call sharing between the two groups of pods.

In addition to the qualitative repertoire differences, there seemed to be differences in call frequency between pods that shared a major part of their repertoire. Table 15 illustrates call frequency differences between pods AB and AI, as well as between pods AE and AK using the call type frequency index.

Table 15. Similarities and differences in call type frequency indices between pods that share a major portion of their repertoires.

	AB POD	AI POD		AEPOD	AK POD
AKS 11 i	.071	n/a	AKS 01 i	n/a	.301
ii	.036	.010	AKS 02 i	.354	n/a
AKS 13	.096	n/a	ii	.329	n/a
AKS 14	.138	n/a	iii	.145	.010
AKS 17 i	.062	.390	AKS 05	.039	.038
ii	.159	.181	AKS 09 i	.008	.206
iv	.021	.081			

N/A indicates that the calculated average frequency was smaller than the standard error of this average, and therefore the index was not printed here.

The greatest difference in call frequency between AB and AI pod was the use of AKS 17i, which was used more often by AI pod, and AKS 14 and AKS 11i, which were predominantly used by AB pod. The frequency differences between AE and AK pod are prominent in all call types except AKS 05, which is a pure tone call type without a pulse tone component.

This analysis allowed acoustic identification of pods that were present in the vicinity of a remote hydrophone in Knight Island Passage on January 27 and February 1, 1996. Although the recordings were of poor quality, the following call types and the pods that made the call type could be identified:

Table 16. Summary of remote hydrophone recordings.

January 27, 1996	February 1st, 1996
AKS 11 i (AB pod)	AKS 01 ii (AK or AD pod)
AKS 17 i (AB or AI pod)	AKS 09 i (AK pod)
AKS 17 ii (AB pod)	AKS 09 ii (AK or AD pod)
	AKS 17 i (AB or AI pod)

Using differences in call type frequency between pods as a deductive method to determine the probability of the presence of a particular pod indicates that AB pod was the only pod present on January 27th. On February 1st, AK and AD pod were present and probably either AB or AI pod.

Discussion

The preliminary results suggest that resident killer whales in Prince William Sound and adjacent areas use calls in a manner similar to resident killer whales in British Columbia and Washington State. Ford (1989) suggested that the calls are used by members of the same pod to stay in contact when the animals are spread out foraging or when they are socializing with members of

other pods. In addition, the calls are probably associated with assortive mating which is seen as mechanism to avoid inbreeding (Ford 1991).

Because it is not certain that the entire repertoire of each pod has been identified, additional recordings of all pods must be analyzed before further examination of repertoire similarities is completed. Additional recordings are needed of pods that have rarely been recorded alone and of pods infrequently recorded.

The results show a clear repertoire distinction between AB, AI, and AN pod cluster and the AD, AE, and AK pod cluster. In none of the recordings has a pod from one group produced a call type attributed to the other group. However, the number of analyzed recordings of AN pod (now two pods, AN10 and AN20) is considerably lower than those for AB and AI pod. A similar situation exists for the other group, where so far only four recordings of AD pod (now AD5 and AD16 pods) could be included in the analysis. Recordings of AN and AD pod were made during the 1997 field season and better definition of the dialect structure of these pods is expected with additional analysis.

The outlook for the identification of pods through recordings from the remote hydrophone is very promising. Analysis of the two poor quality sample recordings available, yielded pod identification with a high probability of accuracy because of the complementary nature of the qualitative and quantitative components of the analysis. The remote listening station installed during the 1997 field season at a similar location in Knight Island Passage is capable of producing recordings of higher quality than the one previously installed and should increase the accuracy of the identification process.

OVERALL CONCLUSIONS

There was one mortality in AB pod in 1997 and two calves were recruited for a net increase of one individual. The pod currently numbers 24 whales and has shown a net gain of two individuals since 1995. Seven of these whales, the AB25 subpod, apparently continue to travel with AJ pod. Although there are two new reproductive females that recruited their first calves in 1997, social disruption within the pod makes potential for recovery to prespill numbers unlikely in the near future. However, the pod may be at a turning point where growth will continue to occur. Additional mortalities are not expected based on our knowledge of current pod structure, except possibly AB45, who was orphaned at the time of the oil spill and is the last remaining member of his subgroup. All other well-documented resident pods remain stable or increasing. All pods of well-known resident whales were completely photographed in 1997 due to the extended field season and high rate of encounters in the Kenai Fjords region. In addition, whales that had not been photographed in seven years or more, including members of AX pod, were photographed in the Kenai Fjords area. Also in that region, a pod of 21 possible residents, AW pod, that had not been observed previously, was documented by photography.

The rate of encounters with killer whales in Kenai Fjords was higher than any year since systematic work began in the Sound in 1984. This was due in part to the extremely effective sighting network developed in conjunction with the tourboat industry. Additionally, there has been an apparent southwestward shift in the distribution of resident killer whales into the Kenai Fjords area. It has been most noticeable in the past two years during the May through October period, and possibly continuing through the winter months. Resident whales have become far more frequently encountered in the Kenai Fjords region as indicated by our observations and those of the tourboat industry. This may be connected with the hatchery enhancement of chinook and coho salmon runs in this area. Coho and chinook appear to be favored salmon species for resident killer whales. The return of coho salmon to Resurrection Bay was particularly strong in 1997 compared to adjacent regions.

The apparent decline in resident killer whale use of Prince William Sound since the time of the spill (1989) has been confirmed by our examination of the number of "whale days" spent by individual whales in the Sound for each year of the study (1984-1996). In previous years the greatest number of resident whales used the Sound in September, with August the next strongest month. Multi-pod encounters (three or more pods) have also shown a decline over the years in the Sound. In 1997 encounter rates in the Sound for July and August were at an all time low. However, the situation we observed in the Kenai Fjords region in 1997 was reminiscent of pre-oil spill observations of resident whales and resident pods in the Sound.

We suspect that 11 of the 22 original members of the the AT1 transient group are dead. There has been no recruitment within the group since 1984. There were only six encounters with this group in 1997 and a total of six different individuals were photographed. The factors contributing to the decline of the AT1 group and its reduced role in the Prince William Sound ecosystem are unknown, but these changes accelerated after 1989 with the death or emigration of nine individuals. Despite increased field effort in April and May, the number of AT1 group encounters did not increase. The social and genetic isolation of this group, the high levels contaminants in their blubber, and the region wide decline in harbor seals are factors that may be inhibiting recovery. It is conceivable the AT1 population will become extinct.

Examination of the use of Prince William Sound by the AT1 transients indicated a significant decline in "whale days" spent in the area over the years from 1984 to 1996. Not only is the population apparently declining, it appears the remaining whales are ranging further and spending more time out of the Sound. All the AT1 encounters occurred in the Kenai Fjords region in 1997.

In GIS based analysis of historic data (1984-96) found that for both residents and AT1 transients the southwestern region was the most used area of the Sound. However, there was a dichotomy between residents, found mainly in Montague Strait and Knight Island Passage, and transients, found mainly in the narrow bays and passages. This probably reflects dietary preferences, as salmon migrate through Montague Strait and Knight passage, while foraging tactics on pinnipeds appear to require careful searching of areas very close to shoreline, such as in the southwest bays and passages. Two patterns of use were found for resident whales, as some pods used Knight Island Passage more frequently than others. This may reflect pod specific strategies and feeding habits. Contaminant data also suggests the possibility of pod specific feeding habits.

Additional genetic samples have clarified the population separations through additional mtDNA analysis of the entire D loop region of the mitochondrial genome. Each population that we defined based on 14 years of association data can also be defined by a single haplotype, consistent in every individual within the population. In addition, within the Prince William Sound/Kenai Fjords resident population, we found the presence of two haplotypes that are identical to the northern and southern resident haplotypes from British Columbia and Washington State. This suggests that pods from the southern resident population from Washington State/southern British Columbia may have moved north and west and entered our region which was already part of the range of the northern residents. Individuals within pods are consistently a single haplotype, although pods of different haplotypes swim together. This is supported by the by vocal repertoire distinction of Prince William Sound pods into two groups that parallels exactly the genetic separation. Vocal tradition seems to help maintain the separation of the two resident haplotypes. We have found that in the nuclear genome sufficient microsatellite polymorphism exists to investigate general patterns of gene flow, and describe population sub-structuring within populations of killer whales. Microsatellite analysis is currently in progress.

Knowledge of sex ratios of all age classes is required for the construction of accurate life tables, and thus is an important component of population dynamics studies. Similarly, in field studies of social behaviour it is extremely helpful to know the sexes of focal individuals. The development of genetic sexing techniques has expanded our ability to understand population dynamics within populations.

Increased sample size has confirmed the wide variation in contaminant levels found in individual killer whales. Statistical comparison of contaminant levels in selected groups of whales has supported our hypotheses that sex, reproductive status and genealogy are important in determining contaminant levels. Since contaminants apparently are passed to offspring via lactation, first born offspring is likely to have the highest contaminant levels, and recently reproductive females the lowest levels. Contaminant levels in transient whales were much higher than in residents; PCB levels averaged 14 times higher and DDT levels averaged 22 times higher. We are concerned that the high contaminant levels in transient killer whales might have impacts on reproductive success for those populations.

Additional samples are needed from the transient populations and from specific pods and from specific individuals for completion of our planned genetic analysis. Contaminant analysis interpretation would also benefit from additional samples from transient whales and from specific pods. Transients have been encountered so infrequently in recent years that sampling is difficult. Sampling will continue in the summer of 1998.

Recently initiated work on the acoustic separation of pods is using call type and frequency to identify individual resident pods. The unique vocal repertoire of the AT1 transients has already been described (Saulitis 1993). Pods present in the Sound have been identified from recordings collected in February 1996 from a remote hydrophone positioned in Montague Strait. Currently a remote hydrophone is operating in the lower Knight Island Passage/Montague Strait area. An additional remote hydrophone is slated for installation in the Resurrection Bay region as part of a cooperative project with the Seward Sea Life Center. In the future remote hydrophone systems may allow the year-round monitoring of the movements of identifiable killer whales without the constant presence of researchers in the field.

ACKNOWLEDGEMENTS

Funding for work in 1989-1991 and in 1995-97 was provided by the *Exxon Valdez* Oil Spill Trustee Council via the National Marine Fisheries Service. Work in 1984 was supported by Hubbs SeaWorld Research Institute and field work in 1986 was supported by the Alaska Sea Grant Program and the National Marine Mammal Laboratory. Data for killer whale pods in PWS in 1985, 1987, 1988, 1992-94 was provided by the North Gulf Oceanic Society. The Society was funded by private donations, the Alaska State Legislature and various State programs, the Alaska Sea Grant Program, Sail Alaska Foundation, the International Wildlife Coalition, and the American Licorice Company. The Vancouver Aquarium, Canada, provided use of their acoustic laboratory. We wish to thank Shayne MacLellan at the Pacific Biological Station for final identifications on fish scale samples. We also thank John Lyle, Kathy Turco and Olga von Ziegesar who provided field assistance in 1994 and 1995.

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

Many people contributed recordings of killer whales in Prince William Sound and adjacent areas. Special thanks go to Kathy Heise, and Lance Barrett-Lennard for high quality recordings crucial to the success of this study. Photographs and assistance was also provided by NPS biologist Peter Armato and his co-workers at Kenai Fjords National Park

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

LITERATURE CITED

- Addison, R.F. 1989. Organochlorines and marine mammal reproduction. *Can. J. Fish. Aquatic. Sci.* 46:330-338.
- Amos, W., Hoelzel, A.R. 1991. Long-term preservation of whale skin for DNA analysis. *Reports of the International Whaling Commission. Special Issue 13: 99-103.*
- Balcomb, K. C., J. R. Boran and S. L. Heimlich. 1982. Killer whales in Greater Puget Sound. *Reports of the International Whaling Commission 32:681-686.*
- Barrett-Lennard, L.G., Smith, T.G., Ellis, G.M. 1996. A cetacean biopsy system using lightweight pneumatic darts, and its effect on the behaviour of killer whales. *Marine Mammal Science 12:14-27.*
- Barrett-Lennard, L.G., Heise, K.A., Saulitis, E.L., Ellis, G.M., Matkin, C.O. 1995. The impact of killer whale predation on Steller sea lion populations in British Columbia and Alaska. *Report of the North Pacific Universities Marine Mammal Research Consortium, University of British Columbia. 66 pp.*
- Bigg, M. A. 1982. An assessment of killer whale (*Orcinus orca*) stocks off Vancouver Island, British Columbia. *Reports of the International Whaling Commission 32:655-666.*
- Bigg, M. A., G. M. Ellis, J. K. B. Ford and K. C. Balcomb III. 1987. Killer whales: A study of their identification, genealogy and natural history in British Columbia and Washington State. *Phantom Press, Nanaimo, British Columbia 79pp.*
- Bigg, M.A., P.F. Olesiuk, G.M. Ellis, J.K.B. Ford, and K.C. Balcomb III. 1990. Social organization and genealogy of resident killer whales (*Orcinus orca*) in the coastal waters of British Columbia and Washington State. *Reports of the International Whaling Commission. Special issue 12 :386-406.*
- Buchanan, F.C., Friesen, M.K., Littlejohn, R.P., and Clayton, J.W. 1996. Microsatellites from the beluga whale *Delphinapterus leucas*. *Mol. Ecol. 5: 571-575.*
- Calambokidis, J., K.M. Langelier, P.J. Stacey, and R.W. Baird. 1990. Environmental contaminants in killer whales from Washington, British Columbia, and Alaska. Abstract submitted to the Third International Orca Symposium, Victoria, B.C.
- Dahlheim, M.E. 1997. A Photographic Catalog of Killer Whales, *Orcinus orca*, from the Central Gulf of Alaska to the Southeastern Bering Sea. NOAA Technical Report NMFS 131. A Technical Report of the Fishery Bulletin, U.S. Dept of Commerce, Seattle, WA.
- Dahlheim, M.E., D.K. Ellifrit, J.D. Swenson. 1997. Killer whales (*Orcinus orca*) of Southeast Alaska: A Catalogue of Photo-identified Individuals. National Marine Mammal Laboratory, National Marine Fisheries Service, Seattle, WA.
- Dahlheim, M.E. 1994. Abundance and distribution of killer whales, *Orcinus orca*, in Alaska, 1993. Unpubl. Report National Marine Mammal Laboratory. Alaska Fisheries Science Center, NMFS, NOAA, 7600 SandPoint Way, N.E. Seattle, WA 98115.
- Dahlheim, M.E. and C.O. Matkin. 1994. Assessment of Injuries to Prince William Sound Killer Whales in: Thomas Loughlin, ed. *Marine Mammals and the ExxonValdez*, Academic Press.
- Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle.
- Ford, J.K.B. 1984. Call traditions and dialects of killer whales (*Orcinus orca*) in British Columbia. Ph.D. dissertation, University of British Columbia, Vancouver.
- Ford, J.K.B. 1989. Acoustic behaviour of resident killer whales (*Orcinus orca*) off Vancouver Island, British Columbia. *Can. J. Zool. 67:727-745.*

- Ford, J.K.B., Ellis, G.M., Balcomb, K.C. 1994. Killer Whales: The Natural History and Genealogy of *Orcinus orca* in British Columbia and Washington State. University of British Columbia Press, Vancouver. 102 pp.
- Ford, J.K.B. 1991. Vocal traditions among resident killer whales (*Orcinus orca*) in coastal waters of British Columbia. *Canadian Journal of Zoology* 69:1454-1483.
- Ford, J.K.B., and A.B. Hubbard-Morton. 1990. Vocal behavior and dialects of transient killer whales in coastal waters of British Columbia, California, and southeastern Alaska. Abstract submitted to the Third International Orca Symposium, Victoria, B.C., Canada.
- Goley, P.D., Straley, J.M. 1994. Attack on gray whales (*Eschrichtius robustus*) in Monterey Bay, California, by killer whales (*Orcinus orca*) previously identified in Glacier Bay, Alaska. *Canadian Journal of Zoology* 72: 1528:1530.
- Hauge, S.Y., Litt, M. 1993. A study of the origin of 'shadow bands' seen when typing dinucleotide repeat polymorphisms by the PCR. *Hum. Molec. Gen.* 4: 411-415.
- Heise, K., Ellis, G., Matkin, C. 1991. A Catalogue of Prince William Sound Killer Whales. North Gulf Oceanic Society, Homer, Ak. 51 pp.
- Helle, E., M. Olsson, and S. Jensen. 1976. PCB levels correlated with pathological change in seal uteri. *Ambio* 5: 261-263.
- Krahn, M.M., G.M. Ylitalo, J. Buzitis, C.A. Sloan, D.T. Boyd, S.L. Chan, and U. Varanasi. 1994. Screening for planar chlorobiphenyls in tissues of marine biota by high-performance liquid chromatography with photodiode array detection. *Chemosphere*. 29:117-139.
- Innis, M.A., Gelfand, D.H. 1990. Optimization of PCRs. *In PCR Protocols*. Edited by Innis, M.A., Gelfand, D.H., Sninsky, J.J., and White, T.J. Academic Press, Inc. N.Y. pp. 3-12.
- Iwata, H.S., S. Tanabe, N. Sakai, and R. Tatsukawa. 1993. Distribution of persistent organochlorines in the oceanic air and surface seawater and the role of ocean on the global transport and fate. *Environ. Sci. Technol.* 27:1080-1098.
- Iwata, H.S., S. Tanabe, N. Sakai, A. Nishimura and R. Tatsukawa. 1994. Geographical distribution of persistent organochlorines in air, water, and sediments from Asia and Oceania, and their implications for global redistribution from lower latitudes. *Environmental Pollution* 85: 15-33.
- Leatherwood, S., Kenneth C. Balcomb III, Craig O. Matkin, and G. Ellis. 1984. Killer whales (*Orcinus orca*) in southern Alaska. Hubbs Seaworld Research Institute Technical Report No.84-175. 54pp.
- Leatherwood, S., C.O. Matkin, J.D. Hall, and G.M. Ellis. 1990. Killer whales, *Orcinus orca*, photo-identified in Prince William Sound, Alaska, 1976 through 1987. *Canadian Field-Naturalist* 104(3): 362-371.
- Leatherwood S., Bowles A.E., Kryieger E., Hall J.D. & Ingell S. 1984b. Killer whales (*Orcinus orca*) of Shelikof Strait, Prince William Sound, Alaska and Southeast Alaska: A Review of Available Information, *Rep. Int. Whaling Comm.* 34: 521-53.
- Matkin, C.O., G.M. Ellis, P. Olesiuk, E.L. Saulitis. in prep. Association patterns and genealogies of resident killer whales (*Orcinus orca*) in Prince William Sound, Alaska. Submitted to *Fisheries Bulletin*, December 1987.
- Matkin, C.O., Matkin, D.R., Ellis, G.M., Saulitis, E. and McSweeney, D. 1997a. Movements of resident killer whales in Southeastern Alaska and Prince William Sound, Alaska. *Marine Mammal Science*, 13(3):469-475.
- Matkin, Craig O., Scheel, D., G. Ellis, L. Barrett-Lennard, E. Saulitis. 1997b. Comprehensive killer whale investigation, *Exxon Valdez Oil Spill Restoration Project Annual Report* (Restoration Project 96012), North Gulf Oceanic Society, Homer, Alaska.

- Matkin, C.O., G.E. Ellis, M.E. Dahlheim, and J.Zeh. 1994. Status of killer whale pods in Prince William Sound 1984-1992. in: *Marine Mammals and the Exxon Valdez*, Thomas Loughlin, ed., Academic Press.
- Matkin C.O., Saulitis E.L. 1994. Killer Whale (*Orcinus orca*) Biology and Management in Alaska, NGOS Report, prepared for the Mar. Mamm. Comm., Contract Number T75135023.
- Matkin, C.O. 1994. The Killer Whales of Prince William Sound. Prince William Sound Books, Valdez, Alaska, 103 pp.
- Morton, A.B. 1990. A quantitative comparison of the behavior of resident and transient forms of killer whale off the central British Columbia coast. Reports of the International Whaling Commission Social Issue 12: 245-248.
- Olesiuk, P.F., M.A. Bigg and G.M. Ellis. 1990. Life history and population dynamics of resident killer whales (*Orcinus orca*) in the coastal waters of British Columbia and Washington State. Reports of the International Whaling Commission Special Issue 12 :209-244.
- Palsbøll, P.J., Vader, A, Bakke, I, Raafat El-Gewely, M. 1992. Determination of gender in cetaceans by the polymerase chain reaction. *Can. J. Zool.* 70: 2166-2170.
- Queller, D.C.; Strassmann, J.E., Hughes, C.R. 1993. Microsatellites and kinship. *Trends Ecol. Evol.* 8: 285-288.
- Richard, D.R., McCarrey, W.W., Wright, J.M. 1994. DNA sequence from the SRY gene of the sperm whale (*Physeter macrocephalus*) for use in molecular sexing. *Can. J. Zool.* 72:873-878.
- Saulitis, E.L. 1993. The behaviour and vocalizations of the "AT" group of killer whales (*Orcinus orca*) in Prince William Sound, Alaska. MS. Thesis, University of Alaska Fairbanks, 193 pp.
- Saulitis, E.L., C.O. Matkin, K. Heise, L. Barrett Lennard, and G.M. Ellis. in prep. Foraging strategies of sympatric killer whale (*Orcinus orca*) populations in Prince William Sound, Alaska. Submitted to *Marine Mammal Science*, January 1998.
- Schlötterer, C., Amos, W., Tautz, D. 1991. Conservation of polymorphic simple sequence loci in cetacean species. *Nature* 354:63-65.
- Sokal, R.R. and F.J. Rohlf. 1995. *Biometry*. W.H. Freedman and Company, New York.
- Strager, H. 1995. Pod specific call repertoires and compound calls of killer whales, *Orcinus orca*, Linnaeus, in waters of Northern Norway. *Can. J. Zool.* 73: 1037-1047.
- Swofford, D.L., Olsen, G.J., Waddell, P.J., Hillis, D.M. 1996. Phylogenetic Inference. In *Molecular Systematics*, Second Edition. Edited by Hillis, D.M., Moritz, C., Mable, B.K. Sinauer Associate, Inc., Sunderland, MA. pp. 407-514.
- Valsecchi, E., Amos, W. 1996. Microsatellite markers for the study of cetacean populations. *Mol. Ecol.* 5: 151-156.
- Zenkovich, B.A. 1938. On the Kosatka or whale killer (*Grampus orca*) *Priroda* 4:109-112 (Translated by L.G. Robbins).
- Zimmerman, S. 1991. A history of marine mammal stranding networks in Alaska with notes on the most commonly stranded cetacean species, 1975-1987. in: *Marine Mammal Strandings in the U.S. Proceedings of the 2nd Marine Mammal Stranding Workshop*.

Appendix 1

DISTRIBUTION OF KILLER WHALE PODS IN PRINCE WILLIAM SOUND, ALASKA OVER A THIRTEEN-YEAR PERIOD, 1984-1996

David Scheel, Craig O. Matkin, and Eva Saulitis

DISTRIBUTION OF KILLER WHALE PODS IN PRINCE WILLIAM SOUND, ALASKA
OVER A THIRTEEN-YEAR PERIOD, 1984-1996

D. Scheel¹, Craig O. Matkin², & Eva Saulitis²

¹Prince William Sound Science Center, Box 705, Cordova, Alaska 99574

²North Gulf Oceanic Society, Box 15244, Homer, Alaska 99603

Please direct correspondence to:

David Scheel

Biology Department, St. Lawrence University

Canton, NY 13617

e-mail: dsc2@music.stlawu.edu

telephone: 315-229-5712

To be submitted to: Marine Mammal Science

Key words: Killer whale, Orcinus orca, area use, Alaska, Prince William Sound

Printed 29 March 1998

ABSTRACT

Thirteen years of encounter data (1984 - 1996) were used to examine killer whale distribution within the Sound. After correcting for search effort (based on kilometers of boat survey per year), we identified four patterns of area use, two among resident pods, and two among transient groups. Area use was similar in resident pods AB, AE, AI, and AN, which all tended to use Knight Island passage and Knight passage more than other areas of the Sound. This pattern was different from that of resident pods AJ and AK, which used all areas of the Sound more evenly. The transient groups made relatively common use of the southwest bays and passages. The AT-1 group was also biased towards the use of mid- and eastern-Sound waters more than any other group, while GOA transients were more frequently found in Montague Strait or just outside the Sound. Despite these differences, Knight Island and Knight passages were among the most used areas for all groups. An examination of foraging behavior indicated that, for the AT-1 group, transient nearshore foraging (likely for pinniped prey) was most common in the areas used most. Foraging behaviors for GOA transients and all resident pods did not differ significantly in different areas of the Sound. The dichotomy between residents, in Montague Strait and Knight Island Passage, and transients, in the narrow bays and passages, reflects dietary preferences, as salmon migrate through Montague Strait and Knight passage, while foraging tactics on pinnipeds appear to require careful searching of areas very close to shoreline, such as in the southwest bays and passages. The different patterns of use between AB, AE, AI, and AN pods on one hand, and AJ and AK pods on the other, did

not fall out exactly along pod lineages as reflected by haplotype and acoustic data, and the reasons for these distinct patterns of area use are currently unclear.

INTRODUCTION

Two distinct types of killer whales (Orcinus orca), termed "resident" and "transient", have been described in the North Pacific. Specializations in foraging behavior appear to be central to understanding differences between the two types. Resident and transient whales differ in almost every known aspect of their ecology. Resident whales eat fish and vocalize frequently while foraging, while transient whales eat marine mammals and are nearly silent while foraging (Morton 1990, Barrett-Lennard et al. 1996, Ford et al. in press, Saulitis in prep). Along the western coast of North America, resident whales are commonly seen in the spring to fall, feeding on salmon, but are seen irregularly in winter; while transient whales may be seen throughout the year but seldom remain at one locality for very long (Heimlich-Boran 1988, Morton 1990). Resident social structure is rigidly matrilineal and both male and female offspring remain with their mothers for life; while transient social structure is more fluid and remains to be completely described (Bigg et al. 1990). The two types do not associate or interbreed, and may be distinguished by mitochondrial DNA (Hoelzel and Dover 1990).

Differences in foraging behavior and social structure of these whale types have been well described. In some cases, differences in habitat use between resident and transient whales have been noted. In addition to the seasonal differences mentioned above, Heimlich-Boran (1988) notes that resident whales in Georgia Strait, British Columbia, usually travel from headland to headland and forage over high relief subsurface topography, while transient whales frequently entered bays and foraged in shallow protected areas, reflecting different strategies for the pursuit of salmon versus harbor seal prey. However, given the central importance of

foraging ecology to this species, relatively little attention has been given to differences in habitat use, particularly of foraging whales.

In Prince William Sound, Alaska, thirteen resident pods (approximately 278 individuals), along with at least two assemblages of transient whales, the AT-1 and Gulf of Alaska (GOA) transients (approximately 55 individuals) have been identified as regular to rare visitors (Matkin et al. 1997). The distribution of killer whale pods in Prince William Sound has been previously discussed in Hall (1986) based on two years of aerial and surface vessel surveys (1976 & 1977). An additional thirteen years of data are now available, based on surface vessel surveys and photographic identification. We present this information here, with particular emphasis on contrasting patterns of area use between resident and transient whales and consideration of change in use patterns over time. We also discuss whether area use and changes over time reflect changes in the availability of prey.

METHODS

The observations reported here are based on identification photographs and behavioral records made between 1984 and 1996, primarily from April to October, over an area of approximately 3500 square kilometers in Prince William Sound, Alaska. Killer whales were located by visual searches, by acoustical monitoring and by soliciting VHF radio reports from other vessels. Whales were individually identified through port-side dorsal fin and saddle patch photography (Bigg et al. 1986). Whales were grouped in pods as defined by Bigg et al. (1990) and Matkin et al. (in review). Group size at each encounter with whales was estimated as the total known size of a pod for well-documented resident pods AB, AE, AI, AJ, AK, AN,

AN10, AN20. Associations among members of resident pods are extremely strong (Matkin et al. in review). For that reason, in encounters with these well documented resident pods, if all known members of the pod were not located during a single encounter, any missing animals were assumed to be nearby. When the group size was less certain in the cases of poorly documented resident pods or transient groups, the number of whales photographed was used as the best estimate of group size. This may have under-estimated numbers in encounters, if some whales were missed by the photographers (more likely with larger groups). Records were entered into a GIS database containing paths of search vessels while looking for whales and the paths of whales during encounters. Details of the database were included in Matkin et al. (1997b). Data were entered and error checked by ES, who was present in the field for most years of the study.

Encounter rates corrected for search effort

Search effort was measured as kilometers that each vessel traversed. To examine search effort, we divided the study area into seven zones (Fig. 1), based on the distribution of search effort. Areas of sparse search effort were made into larger zones to increase the sample size of encounters within a zone. We then tabulated the number of encounters in each year that started within each zone. This number of encounters, divided by the kilometers of effort within that zone, was the encounters-per-unit-effort. This was an indicator of the ease of finding whales in a particular location, and was assumed to indicate how much whales used different areas of the Sound.

Our analyses of area use consider the transient AT-1 group separately from other transients, collectively known as the Gulf of Alaska (GOA) transients. There were not sufficient data to consider the behavior of each GOA group separately. For resident pods, we chose to limit our analyses to pods with more than 50 encounters over the thirteen year study period, in order to retain statistical power, and thus examined the distribution of the six most frequently encountered resident pods (AB, AE, AI, AJ, AK and AN), the latter of which split into two pods, AN10 and AN20, in 1991 but for analyses here is considered a single group. We calculated whale encounter rates per unit effort by year and by map zone, and also compared the period 1984-1989 (hereafter referred to as the 1980s) with 1990-1996 (referred to as the 1990s). Note that the 1989 field season occurred after the eleven-million gallon *Exxon Valdez* crude oil spill in March of that year, so that data from that year, although included in the 1980s, was collected post-spill. However, during the spring and summer of 1989, killer whales were encountering oil in the Sound, and thus 1989 is a transition year between pre- and post-spill behavior patterns. We considered the 1980s to reflect pre-spill behavior and the 1990s to reflect post-spill patterns. We evaluated the distribution of social groups across map zone and decades using a multi-variable analysis of covariance (MANCOVA) with Wilk's lambda as the test statistic, and kilometers-of-effort as a covariate to account for search effort. For groups where encounter rates differed significantly by zone, we used a post-hoc Tukey's Honestly Significant Differences multiple comparisons test to identify differences.

Foraging behavior

Whales were followed and their behavior recorded as opportunity permitted. Behaviors were recorded (following Saulitis et al in prep) as Travel (movement on a consistent compass course, group members surfaced and dove synchronously), Rest (slower than normal movement, maternal units were in close association (< 1 body length from neighbors) and synchronous in movement and breathing), Social (interaction between individuals, including sexual behaviors, chasing, rolling. Breaching, spy-hopping, fluke and flipper slapping were common), or Foraging (any activity related to search for, pursuit of, capture and consumption of prey). Foraging was broken down into sub-behaviors: Feeding (prey seen in the mouth of a whale or surface indications of prey such as blood, grease, or fish scales), and for resident pods, Forage-Resident (tight circling, rapid erratic movement, and lunges often accompanied by frequent vocalization), or for transient whales, Forage-Offshore (milling or slow travel when at the surface ≥ 1 km offshore, silent dives of ten or more minutes duration and underwater movements of ≥ 1 km between surfacing) and Forage-Nearshore (movement following contours of the shoreline often within 20 m of shore, and entering small bays, narrow channels, and exploring rock outcrops or shoal areas). Because Foraging sub-behaviors were not reliably distinguished in the field before 1987, we restricted analyses of behavior to 1987 and later years. Although the path of each encounter and the duration of behaviors were recorded, the specific locations of different behaviors were not. The distance traveled with whales was tabulated from the GIS database, and the zone in which $> 50\%$ of this distance occurred was designated the major zone for that encounter (encounters where no single zone contained $> 50\%$ of the path length were designated as major zone 9, a separate

classification). Behaviors were analyzed based on major zone and for the periods 1987-1992 and 1993-1996 (it was necessary to combine years because of small sample sizes). Pods that did not differ in their area use patterns were combined for analyses of behavior (AB, AI, AN, and AE were considered together, as were AJ and AK). We calculated the proportion of the encounter duration that was spent in Foraging activities other than Feeding. These proportions were arc-sin transformed and their distribution analyzed by time period and major zone using ANOVAs on the arc-sin transformed proportions.

RESULTS

Dedicated boat-based killer whale surveys resulted in a total of 1508 boat-days of search effort and 663 encounters with 19 different killer whale groups over thirteen years (Table 1a). The most intense searching was conducted in Montague Straight and Knight Island Passage (zones 1 and 2 in Fig. 1) while the eastern and outer areas of the Sound (zones 4, 6 and 7) received relatively sparse coverage (Table 1b). Encounters involving the six most commonly sighted resident pods, the AT-1 group or any of the GOA transient groups made up 96% ($N = 638$ encounters) of all encounters (Table 2) and we restricted our analyses to these encounters and groups.

Distribution of killer whales

Over all resident pods and transient groups, encounters increased with search effort and were affected by zone and decade (Table 3, over-all MANCOVA). Average encounter rates varied greatly year to year (Fig. 2), but were generally higher in the 1980s than the 1990s for

AT-1 group, while the reverse was true for AE pod (Table 3, univariate results). For AT-1 group, encounter rates during the 1980s were as low as 0.1 per 100 km searched in 1984, but were more than twice that in 1985, 1988, and 1989 (Fig. 2). In contrast, encounter rates for this group were below 0.1/100 km searched in 1991, 1994, and 1996; and did not rise above 0.2/100 km searched at any time during the 1990s. Overall, encounter rates with AE pod were lower than with the AT-1 groups, but in the 1980s there were only two years when AE pod was encountered more often than 0.01/100 km searched (1985 and 1986), while encounters were at least that high in six of seven years in the 1990s (Fig. 2). Differences between decades were not significant for the combined GOA groups or for any other resident pods.

Area use was similar in resident pods AB, AE, AI, and AN (Table 3), which all tended to use Knight Island passage and Knight passage (zones 1-2) more than other areas of the Sound (Fig. 1 & 3). This pattern was different from that of resident pods AJ and AK (Table 3), which used all areas of the Sound more evenly (Fig. 3).

These two patterns of area use by resident pods differed from those of the AT-1 transient group or the combined GOA transients, which used a larger portion of Prince William Sound resident pods AB, AE, AN, or AI and were more likely to be encountered in the southwestern bays and passages (zone 3) than were any resident pods. The AT-1 group was also biased towards the use of mid- and eastern-Sound waters (zones 6 and 7. Fig. 3) more than any other group, while GOA transients were more frequently found in Montague Strait or just outside the Sound (zones 4 and 5. Fig. 3).

Distribution of foraging behaviors

For the analysis of foraging behaviors, pods AB, AI, AN and AE (hereafter AB-clan+AE) were considered together as these pods showed similar movement patterns. Forage-Resident made up a greater proportion of AB-clan+AE sample time in the period 87-91 than in the period 92-96, but there were no significant differences between major zones in the incidence of this foraging behavior (Fig. 4. ANOVA: time period, $df = 1$, $F = 6.92$, $p = 0.009$; major zone, $df = 7$, $F = 1.39$, $p = 0.209$). We also lumped pods AJ and AK (hereafter AJ+AK) for this analysis, and found no significant difference in the occurrence of Forage-Resident for AJ+AK by either time period or major zone (Fig. 4. ANOVA: time period, $df = 1$, $F = 2.25$, $p = 0.136$; major zone, $df = 7$, $F = 0.28$, $p = 0.961$).

In contrast, for the AT-1 group the occurrence of Forage-Nearshore was significantly greater on encounters that occurred predominantly in zones 1 and 3 and less on encounters predominantly in zones 2 and 5 (Fig. 4), although no differences were found for this behavior by time period. There were no significant differences in the occurrence of Forage-Offshore across major zones or time periods for this group (ANOVAs: Forage-Nearshore time period, $df = 1$, $F = 0.18$, $p = 0.670$; major zone, $df = 7$, $F = 2.16$, $p = 0.043$; Forage-Offshore time period, $df = 1$, $F = 0.01$, $p = 0.923$; major zone, $df = 7$, $F = 0.65$, $p = 0.710$).

Finally, for the GOA groups, behavioral data were sparse, and neither foraging behavior was significantly different across major zones or time periods (ANOVAs: Forage-Nearshore time period, $df = 1$, $F = 0.05$, $p = 0.830$; major zone, $df = 7$, $F = 1.55$, $p = 0.239$; Forage-Offshore time period, $df = 1$, $F = 2.15$, $p = 0.167$; major zone, $df = 7$, $F = 0.45$, $p = 0.835$).

DISCUSSION

Although most individuals considered in this study are known only from Prince William Sound and surrounding waters, the Sound itself is a relatively small area for killer whales to transit. The Sound is 120 km (60 miles) at its widest, and resident killer whales are known to have ranges in excess of 750 km (individuals known from Johnstone Strait, British Columbia or from Prince William Sound each have been recorded in southeast Alaska, in either case a distance of approximately 750 km. Biggs et al. 1990, Matkin et al. 1997). It is therefore not surprising that every group examined was seen at least occasionally in virtually every zone in Prince William Sound, and that each of these groups has also been identified to the west of the Sound in Kenai Fjords. Within the Sound, zones 1 & 2 were among the most used areas for all groups (Fig. 3).

The frequency of encounters for most whales did not change much between the 1980s (1984-1989) and the 1990s (1990-1996). Differences were found only for the AE pod, which was more frequently encountered in the 1990s and for the AT-1 group, which became less frequently sighted in the 1990s. However, our analyses examined only the frequency of encounters, and did not consider the group size during each encounter. A separate analysis of the use of the Sound by killer whales (Matkin et al. 1998) indicates that the number of resident whales encountered per field day declined in 1989, possibly as a result of mortality in the AB pod (the most frequently encountered resident pod) and less frequent encounters with large pods seen more frequently in the 1980s. Matkin et al. 1998 also found that the number of transient whales encountered per field day has declined steadily over the entire 13-year study. In this analysis, we failed to detect the change in AB pod or other resident groups but detected

the decline in AT-1 group. This suggests that AB pod may continue to use the Sound as much as previously, but that the number of resident whales present in an average encounter has declined; while AT-1 group is apparently using the Sound less often in the 1990s than in the 1980s.

We found four patterns of spatial use among eight different resident pods and transient groups. A major difference was apparent between resident and transient-type whales. This partitioning of habitat between residents which occurred in the main entry waterways of the western Sound (Montague Strait and Knight Island Passage), and transients which were more often found in the narrow bays and passages (zone 3), reflects dietary preferences. Salmon migratory pathways enter the Sound at Montague Strait and run up the western side of Knight Island, and resident whales feed on these fish across the width of these channels (Saulitis et al. in prep). However, foraging tactics on pinnipeds appear to require careful searching of areas very close to shorelines (Saulitis et al. in prep), perhaps because pinnipeds are vulnerable as they enter or leave haul-out sites. Data on the distributions of salmon, pinniped, or cetacean prey within the Sound have yet to be published. However, our analyses of foraging behavior were consistent with the interpretation that killer whale distributions reflect their foraging needs, at least in some cases. For example, Forage-Nearshore for the AT-1 group was significantly more common in the southwest bays and passages (zone 3) and along the western side of Knight Island (zone 1), the same areas where this group spends a disproportionate amount of its time (Fig. 3). No similar pattern emerged for Forage-Offshore behavior. For GOA transients as well as for the resident groups, foraging behaviors were also no more likely in the most commonly utilized zones than they were elsewhere. Thus, while

foraging behavior may account for the difference in area use between AT-1 and GOA transients, we found no evidence that they account for differences between the AB-clan+AE pods and AJ+AK pods. It is also possible that a decline in prey availability accounts for the general decline in encounter rates with the AT-1 group. These whales appear to use the Sound heavily for foraging, as the frequency of Forage-Nearshore is highest for AT-1 in the areas where they most often occur. Forage-Nearshore is exhibited when whales are hunting pinniped prey, primarily harbor seals (Saulitis in prep). The trend in harbor seal population for Prince William Sound declines over the period from 1989 to 1995 (Frost et al. 1996), much as does the encounter rate with the AT-1 group.

LITERATURE CITED

- Barrett-Lennard, L. G. B., J. K. B. Ford and K. A. Heise. 1996. The mixed blessing of echolocation: differences in sonar use by fish-eating and mammal-eating killer whales. *Animal Behaviour* 51(3):553-565.
- Bigg, M.A., G. M. Ellis and K. C. Balcomb III. 1986. The photographic identification of individual cetaceans. *Whalewatcher: Journal of the American Cetacean Society* 20(2):10-12.
- Bigg, M. A., P. F. Olesiuk, G. M. Ellis, J. K. B. Ford and K. C. Balcomb III. 1990. Social organization and genealogy of resident killer whales (*Orcinus orca*) in the coastal

waters of British Columbia and Washington State. Report of the International Whaling Commission (Special Issue 12):383-405.

- Ford J. K. B. 1991. Vocal traditions among resident killer whales (*Orcinus orca*) in coastal waters of British Columbia. *Canadian Journal of Zoology*. 69:1454-1483.
- Ford J. K. B., G. M. Ellis, L. G. Barrett-Lennard. In press (Feb 1998). Diet specialization in two sympatric populations of killer whales (*Orcinus orca*) in Coastal British Columbia and adjacent waters. *Can J. Zool.*
- Frost, K.J., L.F. Lowry, R.J. Small, S.J. Iverson. 1996. Monitoring, habitat use, and trophic interactions of harbor seals in Prince William Sound. *Exxon Valdez Oil Spill Restoration Project Annual Report (Restoration Project 95064)*, Alaska Department of Fish and Game, Division Wildlife Conservation, Fairbanks, Alaska.
- Hall, J. D. 1986. Notes on the distribution and feeding behavior of killer whales in Prince William Sound, Alaska. Pages 69-83 in B. C. Kirkevold and J. S. Lockard, eds. *Zoo Biology Monographs, Volume 1: Behavioral biology of killer whales*. Alan R. Liss, New York.
- Heimlich-Boran, J. R. 1988. Behavioral ecology of killer whales (*Orcinus orca*) in the Pacific Northwest. *Canadian Journal of Zoology* 66:565-578.
- Hoelzel, A. R., and A. G. Dover. 1991. Genetic differentiation between sympatric killer whale populations. *Heredity* 66:191-195.
- Jurk, H., L. G. Barrett-Lennard, J. K. B. Ford, E. Saulitis, C.O. Matkin, & K. Heise. 1998. Clan structure of resident killer whales (*Orcinus orca*) in Prince William Sound Alaska

and adjacent areas: acoustic and genetic evidence (Abstract). World Marine Mammal Conference, Monaco.

Matkin, C. O., G. M. Ellis, M. E. Dahlheim, and J. Zeh. 1994. Status of killer whales in Prince William Sound, 1985-1992. Pages 141-162 in T. R. Loughlin, editor. Marine mammals and the Exxon Valdez. Academic Press, San Diego, CA.

Matkin, C. O., D. R. Matkin, G. M. Ellis, E. Saulitis and D. McSweeney. 1997. Movements of resident killer whales in southeastern Alaska and Prince William Sound, Alaska. Marine Mammal Science 13(3):469-475.

Matkin, C. O., D. Scheel, G. Ellis, L. Barrett-Lennard, E. Saulitis. 1998. Comprehensive killer whale investigation. Exxon Valdez Oil Spill Restoration Project Annual Report (Restoration Project 97012), North Gulf Oceanic Society, Homer, Alaska.

Matkin, C.O., G.M. Ellis, P. Olesiuk, E.L. Saulitis. in review. Association patterns and genealogies of resident killer whales (*Orcinus orca*) in Prince William Sound, Alaska. Submitted to Fisheries Bulletin, December 1997

Morton, A. B. 1990. A quantitative comparison of the behavior of resident and transient forms of the killer whale off the central British Columbia coast. Report of the International Whaling Commission:245-248.

Saulitis, E., C. Matkin, K. Heise, L. Barrett-Lennard, & G. M. Ellis. *In prep.* Foraging strategies of sympatric killer whale (*Orcinus Orca*) populations in Prince William Sound, Alaska. Marine Mammal Science.

Table 1: (a) Search effort and encounters with killer whales by year and (b) search effort by zone.

(a)			(b)		Area	Km searched	
Year	Boat-days	Km searched ¹	Encounters ²	zone	(Km ²)	Km searched ¹	/Km ²
1984	129	11341	69	1	285	30160	105.8
1985	60	4452	48	2	359	28018	78.0
1986	60	4680	34	3	354	8262	23.3
1987	29	2057	22	4	6404	5817	0.9
1988	68	4316	27	5	2270	29878	13.2
1989	206	16181	88	6	3542	11430	3.2
1990	249	19603	85	7	2179	5887	2.7
1991	188	15651	54				
1992	136	10492	69				
1993	79	5591	40				
1994	87	6321	32				
1995	125	11066	63				
1996	92	7700	32				
Total	1508	119452	663			119452	

¹ Kilometers of search effort by all vessels.

² Encounters with whales.

Table 2: The number of encounters in which each pod was seen, 1984 to 1996 ($N = 638$ encounters, some of which contained multiple pods, as indicated in parentheses).

Pod	N
AT-1	160 (0)
GOA	33 (6)
All residents	461 (241)
AB	220 (174)
AE	145 (79)
AI	168 (143)
AJ	56 (46)
AK	89 (47)
AN	147 (131)

Table 3: Results of a MANCOVA showing overall and univariate effects of map zone and decade on encounters with AT-1 and GOA transient groups and six resident pods (see text for details).

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

¹ Plus indicates that effort was positively correlated with encounter rates. Zone numbers appear ordered from most to least encounters (after effects of effort and decade have been

accounted for); bars connect zones that were not significantly different (Tukey's Honestly Significant Differences multiple comparisons test, HSD in parentheses).

²The effect of effort in each univariate comparison was significant ($F \geq 5.52$, $df = 1$, $p \leq 0.021$) however the individual statistics were not listed to save space.

Figure Legends

Figure 1. Zones of approximately even search effort used for analyses of encounter rates (see also Table 1b). Zone 3 is shaded to make it easier to see. No search effort or encounters occurred in zone 8 and it is therefore excluded from consideration in our analyses.

Figure 2. Encounter rates with AT-1 and GOA transient groups and all resident pods by year, 1984 to 1996.

Figure 3. Encounter rates with AT-1 and GOA transient groups and all resident pods in zones 1 through 7.

Figure 4. The incidence of Forage-Resident behavior by time period and major Zone.

Figure 5. The incidence of Transient Forage-Nearshore and Forage-Offshore behaviors by time period and major Zone.

