Exxon Valdez Oil Spill
Restoration Project

Comprehensive Killer Whale Investigation

Restoration Project 98012
Annual Report

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

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EXECUTIVE SUMMARY

Killer whales were monitored in Prince William Sound, Alaska with funding from the *Exxon Valdez* Oil Spill (EVOS) Trustee Council in 1989, 1990, and 1991 (damage assessment) and in 1993 (restoration monitoring). Monitoring was continued in 1995-1998 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 1998. The goal of the monitoring has been to obtain identification photographs of all whales in all major resident pods including AB pod and the AT1 transient group on an annual basis. Photo-identfication techniques (after Bigg et al. 1990) were used to identify individual whales. The current photographic database includes thousands of frames of film collected from 1984-1998 used to provide individual identifications for each encounter with whales. Vital rates for AB pod and all other frequently sighted resident pods were calculated based on the photographic data.

The total number of whales in well-known resident pods other than AB pod has increased from 66 to 91 whales from 1988 through 1998, while AB pod has declined from 36 whales to 25 whales in that same time period. All resident pods have increased since 1984 except AB pod. From 1995 to 1998 AB pod had a net increase of three individuals, due to recruitment of five calves and two mortalities. Although AB pod numbers are again increasing it would be premature to predict a recovery of this pod. Seven members of the pod (AB 25 subpod) still appear to travel with AJ pod.

Sighting data for the AT1 transient group in 1998 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. We believe that only 11 of the original 22 whales in the AT1 group are still alive. Only eight of the original 22 whales attributed to the AT1 group were photographed in 1998. The rate of encounter with members of this group has also declined significantly since 1989. Modeling of resighting data for the individual AT1 group whales supports 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 1998, including a total of 1,710 boat-days of search effort and 761 encounters with whales. Analysis of spatial distribution over time has been completed and manuscript developed (attached).

Biopsy tissue sampling for genetic analysis and contaminant analysis was continued in 1998 using a biopsy dart system and field techniques developed by Barrett-Lennard et al. (1996). An additional 17 tissue samples from individually identified killer whales were collected in 1998. Of these, 15 contained sufficient blubber for contaminant analysis. Since the beginning of the study, a total of 100 samples have been collected from resident and transient killer whales and used in genetic analysis. Of these, 76 contained adipose tissue was used for contaminant analysis.

The entire mitochondrial DNA D-loop region of the newly-biopsied killer whales was sequenced. Analysis of these sequences refined our understanding of the previously-described genetic divergence between resident- and transient-type killer whales. In 1998 a new transient mtDNA haplotype was discovered in a group of previously unphotographed whales. These transients were most similar to the Gulf of Alaska haplotype and unlike the AT1 group transients. MtDNA analysis supported the existence of two distinct maternal

Eleven of the 22 whales from the transient AT1 group have not been observed or photodocumented for at least 7 years despite extensive field effort. While mortalities in transient groups cannot be confirmed with the same certainty as for residents, it is likely that these whales are dead or have emigrated from the Sound. Most of these whales disappeared in the year following the spill.

The AB pod and AT1 group appear to have been injured due to the effects of the *Exxon Valdez* oil spill. 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-identfication 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 from sites of whale predation when possible, 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 (Saulitis et al. in press).

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 have been incorporated into models of harbor seal population dynamics (project 064, seal trophics). A geographic information system (GIS) database was designed and the data from 1984 to 1998 entered into a computer from hand-written data sheets. Sighting records provide considerable behavioral information (travel rates, duration of feeding bouts, etc.). Location of encounters and basic behavioral information (resting, feeding, traveling, etc.) are available for each sighting.

It has been a goal of the GIS project to provide a systematic and easily accessible storage system for geographically-referenced data generated by this ongoing project since 1984. The system can be used to address questions of interest to restoration management, and to examine the distribution of whale groups over time in Prince William Sound. Data analysis is providing detailed demographics and spatial distributions of resident and transient killer whales.

This project has 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 100 whales. Samples were obtained using lightweight biopsy darts (Barrett-Lennard et al. 1996). The genetic analysis used both mitochondrial DNA (mtDNA) and nuclear DNA microsattelites to separate populations and examine breeding systems. MtDNA evolves quickly, is only passed through the maternal line, and provides a faithful record of female lineages over long periods. MtDNA is considered an appropriate marker for distinguishing well-established populations. Microsatellite analysis is currently being used to examine male mediated breeding patterns and provide further resolution of populations, as well as to describe breeding systems.

Contaminant analysis has been completed on blubber tissue collected simultaneously with the genetic samples. 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)

FIELD METHODOLOGY

Field work for the 1998 photo-identfication 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-identfication was conducted from the skiff while acoustic recordings were made from the R.V. *Lucky Star*. The R.V. *Lucky Star* operated in both the Kenai Fjords and Prince William Sound region. In addition, the R.V. *Whale 2*, a 7.9m, live-aboard vessel powered by a 165 hp diesel engine with inboard/outboard drive operated primarily in the Kenai Fjords region.

N.G.O.S. biologists on the R.V.*Whale 1* (a 7.8 m light motor-sail vessel with 50hp outboard) also photographed killer whales and kept vessel logs and encounter sheets during surveys directed at humpback whale photo-identfication. 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 traveled were recorded. Vessel track was plotted. Record was made of time and location of all whale sightings and weather and sea state noted at regular intervals.

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

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 and labeled with the next available number.

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

Calculation of Vital Rates

Most new calves were already present at the beginning of the field season and exact birth dates could not be determined. We followed the method of Olesiuk et al. (1990) and placed the birth of all calves in January for calculation of vital rates. Thus, birth rates could not be measured, and recruitment rates represent the survival of calves to about 0.5 years of age.

The determination of mothers of new calves was based on the consistent close association of calves with an adult female. 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 press). 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 field 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 for a season or longer; however, this has not been observed for individuals. In a few instances missing whales have been found dead on beaches, but strandings of killer whales are infrequent events and most missing whales are never found. During 1975 to 1987 only six killer whales were found on beaches throughout the entire Gulf of Alaska (Zimmerman 1991). One explanation for the lack of stranded killer whales comes from the observations of early Soviet researchers. Killer whales that were shot for specimens were reported to sink (Zenkovich 1938).

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

Killer whales were encountered on 48 occasions in 1998 (Table 2). Researchers spent approximately 158 hours traveling 1122 km with killer whales.

Table 2. Encounters with killer whales by vessel in 1998

Vessel	# encounters	Distance (km)	Time (hr)
Lucky Star	22	535.8	77.1
Whale 1	2	21.6	3
Whale 2	19	553.8	74.6
Viewfinder	2	3.0	0.7
Fjordland	1	8.3	0.6
Bardy	2	4.0	2.0
Total	**48**	**1126.5**	**158.0**

In 1998 there were forty-one encounters with resident pods. There were four encounters with the AT1 transient group, two encounters with Gulf of Alaska transients and one encounter with possible transients (Table 3).

Despite a similar number of encounters in 1997 (50) and 1998 (48), because of weather and other logistic considerations, 30% more time was spent with killer whales in 1997 (205 hours) than in 1998 (158 hours). Some of the major resident pods, including AJ and AG, were not completely photographed in 1998.

Encounter rates were much lower in Prince William Sound than in Kenai Fjords again in 1998. In Kenai Fjords there were 36 killer whale encounters during 57 vessel days for an average of 0.63 encounters/day compared to an average of 0.79 encounters per day in 1997. In Prince William Sound there were 12 killer whale encounters for 41 vessel days for a average of 0.29 encounters/day compared to an average 0.14 encounters per day in 1997. The overall encounter rate for 1998 of 0.49 encounters per day was higher than the 0.39 encounters per day for 1997. In 1998 all encounters with three or more resident pods ("superpods") occurred in August, September and October (Table 3). Encounters with transient whales were scattered throughout the season, however, there were no encounters with the AT1 transient group after 2 August in 1998.

Table 3. Summary of 1998 encounters with killer whales in Prince William Sound (PWS) and Kenai Fjords (KF)

DAY/MO/YR	BEGIN LOCATION	END LOCATION	PODS	REGION	#WHALES
21/02/98	3 mi S Caines Head	2 mi N Caines Head	AJ, AN20	KF	56
12/05/98	2 mi N Caines Head	5 mi N Caines Head	AI, AB	KF	32
14/05/98	5 mi N Agnes Bay	5 mi E Agnes Bay	AD5	KF	17
12/06/98	off Callisto Head	inside No Name I	AD14, AK	KF	19
18/06/98	off Pleiades Is	mouth of Icy Bay	AD14	KF	7
21/06/98	off N end Matuska Is.	off N end Harbor Is.	AD5	KF	17
22/06/98	1/2 mi N of N end Cheval	3 mi S Mary's B	AD5	KF	17
23/06/98	1/2 mi S of S end Cheval	off Cape Res	AD14	KF	7
23/06/98	off Shelter Bay	N end P of W Pass	AT1	PWS	2
8/07/98	2 mi SW Mary's	2 mi E Barwell	AD14, AK	KF	19
12/07/98	1.5 mi W Point Countess	2 mi S Point Countess	AK	PWS	12
14/07/98	Holgate Head	Aligo Pt	AT1	KF	2
15/07/98	1/2 mi S Toe Pt	5 mi SW S end Granite I	transients?	KF	3
18/07/98	NE end Cheval	NW corner Cheval Is	AD14,AK	KF	19
21/07/98	4 mi N Cheval	2 mi N Cheval	AD14,AK	KF	19
24/07/98	3 mi W Rugged I	bet Barwell and Rugged Is	AT1	KF	6
27/07/98	N end Fox Is	1.5 mi N N end Fox Is	AI,AK	KF	19
29/07/98	3 mi E Pt Grace	bet Green I & Port Chalmer	AE	PWS	16
29/07/98	bet Green I & Port Chalm.	bet Green and Montague	AB	PWS	25
2/08/98	1 mi W McLeod Harb.	2 mi SE Needle	AE	PWS	16
2/08/98	N end Evans Is	2 mi SE Pt Grace	AT1	PWS	3
5/08/98	Aialik Cape	1 mi W Ship Lift	AK	KF	12
5/08/98	1/2 mi W Chat I	Dora Psg	AD14	KF	7
8/08/98	N end Fox Is	2 mi E Barwell	AK	KF	12
9/08/98	3.5 mi SW Rugged	3 mi N Chevall	AK,AD14	KF	19
14/08/98	bet Hogan Bay &Green I.	bet McLeod H. &Danger I.	AB25,AJ,AG	PWS	70
15/08/98	2 mi N slide on Latouche	2 mi S slide on Latouche	AK	PWS	12
15/08/98	Agnes Bay	Inside No Name I.	AJ,AB25,?	PWS	43
18/08/98	off Port Chalmers	3 mi E Rocky Bay	AB,AI,AE	PWS	48
19/08/98	off Rocky Bay	3 mi SW Schooner Rk	AI	PWS	7
19/08/98	1 mi SW LittleGreen I.	2 mi SW Needle	transients?	PWS	4
25/08/98	2 mi E Chevall	1 mi E Caines H	AN10	KF	20
26/08/98	off Porcupine Cove	off Callisto Head	AN10	KF	20
27/08/98	4 mi N Harbor I	1 mi W Coleman	AN10,AB	KF	45
28/08/98	1 mi S Shipyard	1.5 mi SW Sunny Cove	AB,AD14	KF	32
29/08/98	1.5 mi SW of Marys Bay	3 mi E Barwell I.	AB	KF	25
31/08/98	Caines Head	2 mi W Rugged I	AB,AN10	KF	45
6/09/98	Caines Head	3 mi S Miller's landing	AB,AJ	KF	61
7/09/98	Callisto Head	3 mi N Miller's landing	AB,AJ	KF	61
8/09/98	1 mi NE Chevall	3 mi S Callisto	AB,AJ	KF	61
13/09/98	1 mi N 3 Hole Bay	1 mi S Natoa Is	AC20	KF	5
14/09/98	SE Corner Rugged I.	1 mi N Cheval	AB,AJ	KF	61
2/10/98	off Killer Bay	Cape Resurrection	AB,AJ,AN10	KF	81
12/10/98	1 mi S Seward	5 mi S Seward	AK	KF	12
02/12/98	Tonsina Crk, Res Bay	same	AB, AN10	KF	45
06/12/98	Thumb Pt/ Res Bay	1 mi SW Thumb Pt	AB, AN10	KF	45
22/12/98 #1	2 mi S. Caines Head	3 mi. S of Caines Head	AB	KF	25
22/12/98 #2	Caines Head	same	AI	KF	7

Kenai Fjords: 36 encounters/ 57 vessel days; Prince William Sound: 12 encounters/41 vessel days

Figure 2. Number of whales in AB pod and in all other well-documented resident pods, 1984-1998

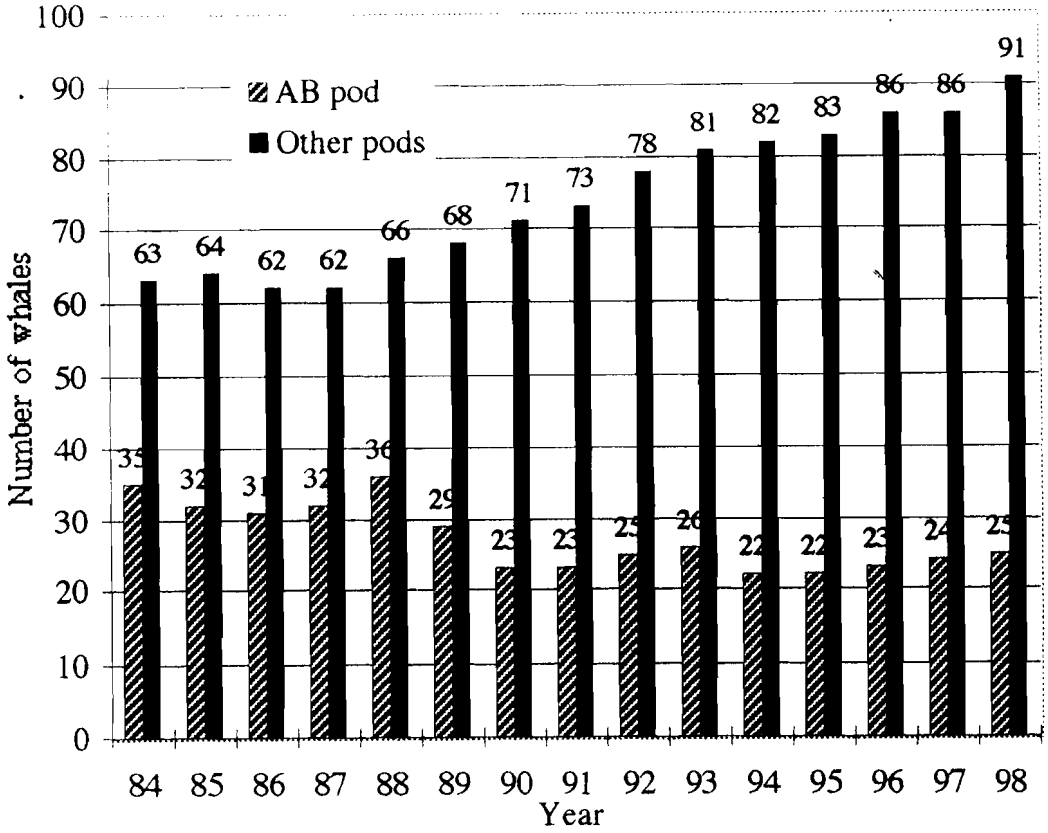


Figure 3. Numbers of whales in well-documented resident killer whale pods 1984-1998

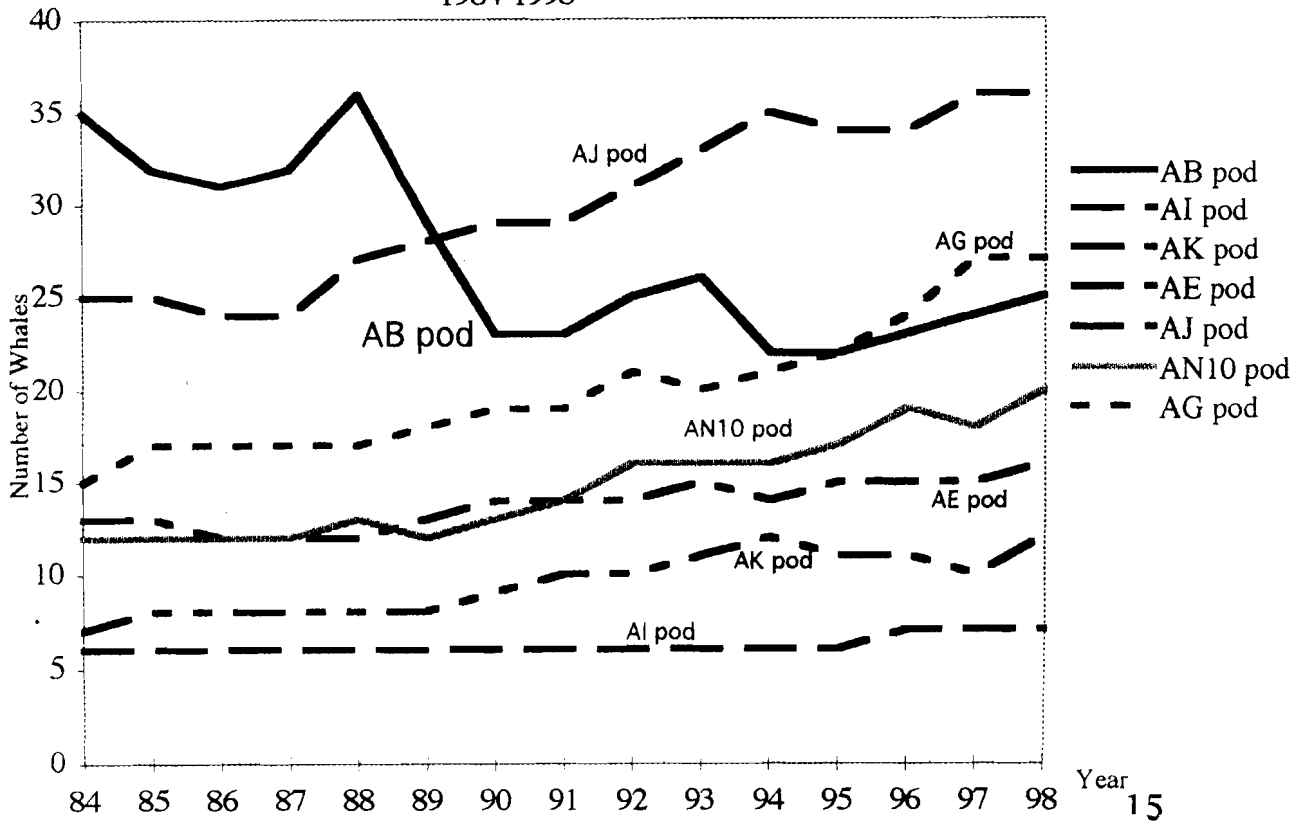


Table 5. Mortality and recruitment rates in Prince William Sound resident pods.

	Recruitment rates in Prince William Sound Resident Pods							
	AB	AI	AK	AE	AJ	AN10	AG	All other than AB,AG
85	0	0	14.3	7.7	0	0	13.3	3.2
86	6.3	0	12.5	0	0	0	0	1.6
87	6.4	0	0	0	0	8.3	0	1.6
88	15.6	0	0	8.3	12.5	8.3	5.9	8.1
89	0	0	0	15.4	3.7	7.7	5.9	4.5
90	0	0	12.5	7.7	3.4	0	11.1	4.4
91	4.3	0	11.1	0	0	7.7	0	2.8
92	8.7	0	0	0	10.3	14.3	10.5	6.8
93	4	0	10	7.1	9.4	0	4.8	6.8
94	3.8	0	9.1	0	5.9	6.7	5	4.9
95	0	0	0	7.1	0	6.3	4.8	2.4
96	9.1	16.7	0	0	0	11.8	9.1	7.9
97	8.6	0	0	0	5.9	5.2	12.5	3.4
98	4.2	14.3	20	6.7	0*	10.5	0	6.8
	Mortality rates in Prince William Sound Resident Pods							
	AB	AI	AK	AE	AJ	AN10	AG	All other than AB,AG
85	8.6	0	0	7.7	0	0	0	1.6
86	9.4	0	12.5	7.7	4	0	0	4.7
87	3.2	0	0	0	0	8.3	0	1.6
88	3.18	0	0	8.3	0	0	5.9	1.6
89	19.4	0	0	8.3	0	7.7	0	3
90	20.7	0	0	0	0	0	5.6	0
91	4.31	0	0	0	0	0	0	0
92	0	0	0	0	3.4	0	0	0
93	0	0	0	0	0	6.3	9.5	2.5
94	19.2	0	0	6.7	0	0	0	2.4
95	0	0	8.3	0	2.8	0	0	2.4
96	4.5	0	0	0	0	0	0	0
97	4.3	0	9	0	0	5.2	0	2.3
98	0	14.3	0	0	0*	0	0	1.2
# in pod84/98	[35/2:	[6/7]	[7/12]	[13/16]	[25/36]	[12/20]	[15/27]	[63/91]
	*entire pod not photographed							

Table 7. Sighting histories for all AT1 transient whales for years with effort greater than 40 days.

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

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

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.

AB pod appeared to be using the Resurrection Bay/Kenai Fjords on a regular basis in the fall and early winter. Regular fall and winter use of this area by resident killer whales, including AB pod has been reported for the past several years. These reports began with the initiation of regular winter tour boat operations in Resurrection Bay. It is conceivable regular winter use of Resurrection Bay/Kenai Fjords has been a long-term pattern that has not been documented until recently. Whales seem to be more difficult to observe in the winter because of sea conditions and possible changes in behavior (i.e. longer downtimes) and they could be easily overlooked by the casual observer.

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

Again, Kenai Fjords had a much higher rate of encounter with killer whales than Prince William Sound, following the pattern first observed in 1997. In part this is due to the efficiency of the observer network made possible by the presence of numerous tourboats in Kenai Fjords. However it is clear that the number of animals in the August period have declined over recent years in the Sound (see Changes in Habitat Use, this report) and subjective accounts from tourboat operators indicate killer whales have been encountered much more frequently in Kenai Fjords in summer over the past two years than in previous years. An increase in resident killer whale sightings has been the reason for this change. We suspect enhanced coho and Chinook salmon returns are in part responsible for the increased use of the Kenai Fjords by resident whales. These fish species are documented prey items.

CHANGES IN HABITAT USE AND GIS PRODUCTS

Introduction

Historical data on killer whales collected by the North Gulf Oceanic Society (NGOS) from 1984 to 1998 includes six years of pre-EVOS data, and provides the best available record of how killer whale habits may have changed following the oil spill. The goal of this project is to provide an geographically-referenced analysis of this data to address questions of interest to restoration management, and to examine the distribution of whale groups over time in Prince William Sound. 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 (to 1996) of data are now available, based on surface vessel surveys and photographic identification. We have used this data in examination of changes in use patterns over time. The GIS portion of the project had three objectives in FY98: (a) Identify critical habitats used by transient killer whales in Prince William Sound; (b) Create a display quality map portraying the critical habitats identified and explaining their importance to transient whales (no costs for production or distribution of this map are included); (c) Draft publication for submission to professional journal.

In this report, the sightability of individual resident and AT1 transient whales also was examined in order to investigate the hypothesis that there was reduced sightability following the 1989 *Exxon Valdez* Oil Spill. The spill was not anticipated, and an

During this fiscal year, the manuscript was sent out for external review to other killer whale biologists. Their comments were taken into account in revising the manuscript. As a result of reviewer's input, we are expanding the scope of the paper with an additional set of data and analyses (revisions currently in progress).

Objective 2 was addressed in a poster presented at the EVOS Legacy of an Oil Spill Ten Years After workshop (Scheel, D., C.O. Matkin, E. Saulitis.1999. Distribution of killer whale pods in Prince William Sound, Alaska,1984-1996.)

Residents

Inspection of the graphic representation of effort and encounter data for resident killer whales confirmed that August was the month of greatest effort and the month with the second highest encounter rate (Figure 5) The means of August daily sightings per hour by the primary research vessel within the years 1984-1990 (except 1987) and within the years 1991-1998 were not significantly different ($p=0.08$ and $p=0.55$ respectively; Figure 6). Pooled means of the number of killer whales encountered per hour in August prior to and including 1990 versus after 1990 (6.39 vs. 2.73) were highly significantly different ($p=0.0007$; Figure 6).

AT1 Transients

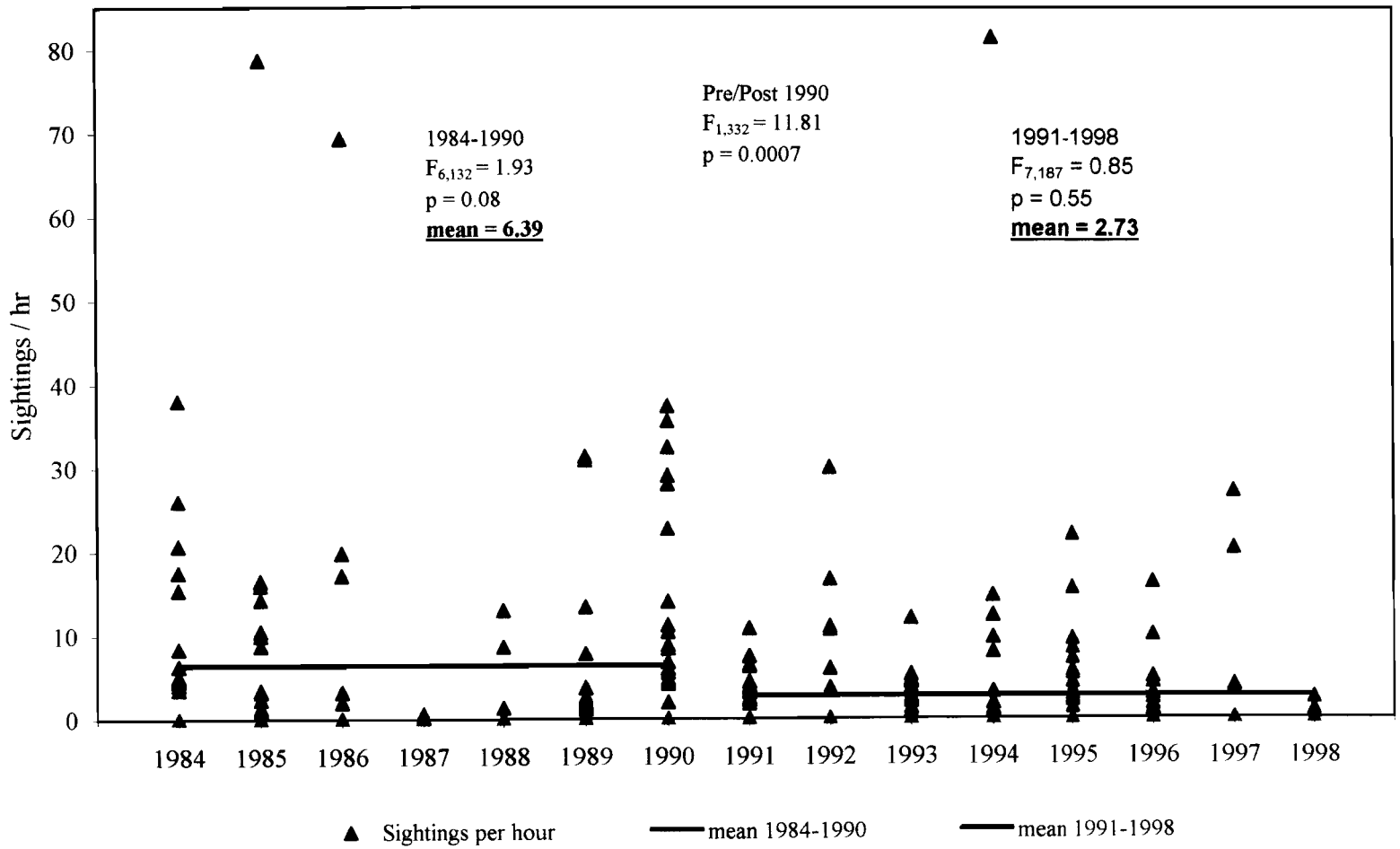
The means of daily sightings by the primary research vessel in the southwestern Prince William Sound between years 1984-1989 (except 1987) and between 1990-1998 were not significantly different ($p=0.91$ and $p=0.11$ respectively; Figure 7). Pooled years means pre and post 1989 were highly significantly different ($p=0.0002$; Figure 7). On average more than 3 times as many individual AT1 transient killer whales were sighted by the primary research vessel before 1990 (0.35/day) than after 1990 (0.12/day).

Discussion

From the onset of the study in 1984, the primary focus of the research has been photoidentification . Data on distribution of killer whales was not collected in a systematic format designed to answer specific questions regarding changes in distribution. Thus, GIS and other techniques of examining distribution are limited. Bias is introduced by such factors as improved ability to find animals with experience and development of a sighting network as well as stochastic forces. The effect of these on sightings per effort are impossible to accurately quantify. With these caveats, the distributional results must be examined with caution both in the GIS based report (attached) and in the other calculations presented here.

To reduce some of the bias in our data, we focused our examination of resident whale use on the month August and refined our measurement of effort to an hourly examination of effort by the single most consistent vessel. Thus, we are confident that the observed decline in number of resident whales per hour using the southwestern Sound is real. More problematic is the observation that the decline did not occur until after 1990. The very large number of vessels involved in oil spill cleanup activities in 1989 and 1990 created a whale sighting network that was much more efficient than for any other years. However, it is unlikely that this completely masked an earlier change in pattern of use by resident whales and we suspect that 1991 was the pivotal year in the decline. Thus, the timing of this change clouds direct linkage to the oil spill. It is not clear whether the observed changes are due to natural factors or factors associated with the spill that were not expressed until after 1990. It is not known whether changes in environmental, social, or other conditions are responsible for the observed changes.

Figure 6. Comparison of resident killer whale sightings per hour for August in southwestern Prince William Sound 1984-1990 and 1991-1998.



The GIS analysis(attached) examined the frequency of encounters (not number of whales) with the major resident pods over the period 1984-1996 for all months and throughout Prince William Sound. It indicated that encounter rates did not change appreciably for this period. In light of this, we suggest that the reduced number of whales using the southwestern Sound in August since 1990 is a result of (1) reduced numbers of whales in AB pod following the spill and (2) reduced number of encounters with resident pods other than the six major resident pods examined in the GIS studies.

The dramatic decline in the number of AT1 individuals sighted after 1989 coincides with the loss of nearly half of the 22 members of this group following the oil spill. Not only are there half as many individuals using the area, but the amount of time the remaining whales are spending in the area seems to be declining. The number of whales per day using the southwestern Sound has declined by almost two thirds since 1989. This may reflect the severe decline of harbor seals in recent years which might requires more extensive foraging or other environmental changes.

POPULATION GENETICS

Introduction

In previous years we have focused our attention in the genetic component of this study on the identification of population subdivision in the killer whales of Prince William Sound. We verified that the resident and transient forms differ genetically, and showed that each is more closely related to whales of the same form in British Columbia than to local members of the opposite form. We also provided evidence that the transient form consists of two subpopulations, which are genetically distinguishable from each other and from the transients of British Columbia. Finally, we showed that the Prince William Sound residents consist of two genetically distinguishable groups, one of which bears a genetic resemblance to the so-called B.C. northern residents, and the other to the B.C. southern residents. All of these results were based on the analysis of maternally-transmitted mitochondrial DNA.

Comparative analyses of mitochondrial DNA provide reliable information regarding population history and patterns of female movements, but do not shed light on gene flow resulting from male movements or from mating between groups during temporary associations. In 1998 we continued to analyze mitochondrial DNA from new samples, but shifted the majority of our attention to the analysis of microsatellite nuclear DNA, which is transmitted through both maternal and paternal lines. This analysis allowed us to ask whether mating occurs between residents and transients, and between subgroups of the two forms. This knowledge is essential for estimating the viability of the killer whale populations of Prince William Sound. If each killer whale form and subpopulation are truly independent, the survival prospects of each should be considered separately.

Data Analysis

Paternity exclusions

We wrote a program that used the microsatellite data to conduct paternity exclusions. The program selected all the biopsied cow-calf pairs (based on Matkin et al., 1999) in the data set, and tested these maternal relationships by confirming that the pair had at least one allele in common at every microsatellite locus. All offspring that passed this test were then matched with possible fathers by removing the maternal contribution to the offspring's genotype, and comparing the resulting paternal contribution to those of adult males. All adult males in the data set of unknown age, or known to have been born at least 13 years before the offspring (based on Matkin et al. 1999) were included in this comparison.

Allele frequency analysis

The microsatellite data were grouped based on population subdivisions suggested by the mitochondrial DNA analyses (Matkin et all. 1998). Wright's F-statistics (Weir and Cockerham 1984) were then estimated for the grouped data using the program FSTAT (Goudet 1994); permutation tests were used to determine the probability that the estimated values differed from zero (which would indicate no population subdivision).

In addition, we calculated Nei's unbiased genetic distances D_s (Nei 1972) between all putative subpopulations using the program MICROSAT (Minch 1995). D_s does not assume any particular mechanism of mutation, unlike recently-developed measures which assume that mutation occurs in a stepwise fashion (eg $\delta\mu^2$, Goldstein et al. 1995). Stepwise mutation-based measures are expected to be linear with respect to time at longer time scales, whereas D_s is considered a more appropriate measure when divergences have occurred recently (Goldstein et al. 1995, Paetkau et al. 1997). The genetic distance matrix was used to construct a UPGMA (unweighted pair group method with arithmetic averaging) phylogram, using the program PAUP (Swofford 1999). This tree indicates the relative similarity of each subpopulation but, since UPGMA is effectively a cluster analysis, it is not an explicit reconstruction of population phylogeny. We have avoided attempting to construct a sub-population phylogeny since the history of the subpopulation divergences is quite possibly reticulate rather than radiational, due to occasional inter-group matings (see Smouse 1998).

Results

Biopsy Samples

Prior to 1998, we had obtained DNA samples from 83 killer whales in and around Prince William Sound. Of these, 77 individuals were biopsy-darted, and 6 were sampled as stranded carcasses. In 1998 we biopsied an additional 17 whales, including three from EVOS-impacted AB pod, three, four and five from AK, AD and AJ pods respectively, and 2 from a previously-unidentified group. This brings our total sample size to 100. For the purpose of comparison, results are also presented from a concurrent genetic study in British Columbia (L.B.L., unpublished data).

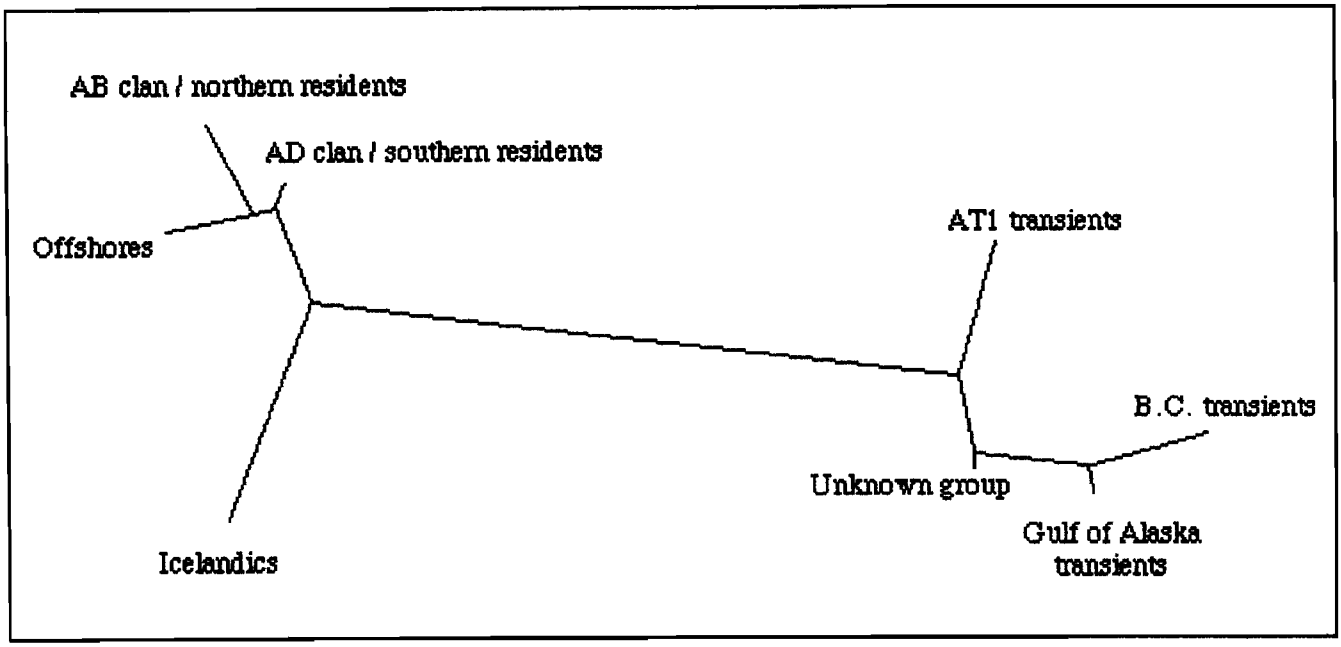


Figure 8. Neighbour-joining tree based on mitochondrial D-loop sequences. A maximum likelihood mutation model based on empirical base frequencies was used as the distance measure. The "unknown group" refers to a group of whales sighted in Prince William Sound in 1998: two individuals that were biopsied had identical D-loop haplotypes. A similar topology was obtained as the consensus of 1000 bootstrapped maximum likelihood trees (not shown, similar to Figure 19 in Matkin et al. 1998). Bootstrap support for the separation of the left and right hand clusters in this tree was greater than 99%, but there was less than 50% support for any particular topology within either cluster.

Microsatellite DNA

Twenty seven sets of microsatellite primers were tested with killer whale DNA under low stringency conditions. Five of the loci failed to amplify, and four amplified but were monomorphic. Seven additional loci amplified fewer than three alleles in the test data set or produced ambiguous bands, leaving 11 readily-scoreable polymorphic loci. All DNA samples were amplified at these 11 loci. For the samples from dart biopsies, the proportion of missing genotypes in the data set was 0.004. For the carcass samples, this proportion was much higher (0.167), presumably because several of the carcasses had severely degraded DNA. None of the 11 loci had allele frequencies that differed significantly from Hardy Weinburg expectations (tested on British Columbian northern residents, L.B.L., unpublished data), indicating that null alleles (Bruford and Wayne 1993) were rare or nonexistent. All loci were equally polymorphic in both males and females, ruling out the possibility of sex linkage.

Table 11. Weir and Cockerham (1984) estimators of F-statistics, for each pair of sampled subpopulations* from Prince William Sound and British Columbia.

BCT					
NR	0.2778				
SR	0.2290	0.1439			
OFF	0.1532	0.2785	0.3206		
PWS	0.2591	0.0752	0.1872	0.3048	
AT1	0.2239	0.4294	0.4293	0.4223	0.3994
GAT	0.0652	0.2510	0.2257	0.1815	0.2338
0.2902					
	BCT	NR	SR	OFF	PWS
AT1					

Note: Subdivided populations are expected to have higher Fst values than non-subdivided ones.
*Subpopulations are abbreviated as follows: British Columbian transients, BCT; British Columbian northern residents, NR; British Columbian southern residents, SR; Prince William Sound residents, PWS; Gulf of Alaska transients, GAT; and the AT1 transients of Prince William Sound, AT1.

Mating patterns in resident killer whales

Four striking patterns emerge from microsatellite DNA analysis regarding mating patterns in Prince William Sound killer whales. Firstly, it is clear from the paternity exclusion tests (Table 2) that resident killer whales seldom if ever mate within their pods, despite the lack of permanent dispersal of either males or females. Matings must occur during temporary associations of pods. This corroborates similar findings for British Columbian resident killer whales (L.B.L., unpublished data). Secondly, there is no evidence that the two genetic and acoustic clans identified by H.J. and L.B.L (acoustics section of this report, AD and AB clans in Figure 2) are a barrier to mating. Thirdly, British Columbian northern residents and Prince William Sound residents are closely related (Table 4, Figure 2), indicating that gene flow occurs between them either directly or via an intermediate population in south eastern Alaska, or else that they have separated from a common population very recently. Fourthly, the non-significant F_{is} value in Table 3 is evidence that killer whale populations are not inbred with respect to their subpopulations.

Separation of resident and transient lineages

The paternity tests and the allele frequency analyses (Tables 3 and 4) present two strong lines of evidence that mating between residents and transients occurs extremely rarely if ever. The significant F_{st} and F_{it} values in table one are also indicative of population subdivision. This finding resolves a long-standing question. We established in previous years that females did not permanently emigrate between resident and transient killer whale forms in Prince William Sound, and preliminary findings of a similar nature regarding British Columbian killer whale populations were made as long ago as the late 1980's (Stevens et al. 1989). However, as mentioned in the introduction, mitochondrial DNA analysis alone could not rule out the possibility of male-mediated gene flow.

Implications for EVOS-impacted killer whales

The AB resident pod of Prince William Sound was severely impacted by *Exxon Valdez* Oil Spill (Matkin et al. 1998, and references therein). The findings to date suggest that this pod is not a genetically discrete unit--in other words, AB-pod calves have fathers from outside the pod. Since the population of resident killer whales in Prince William Sound does not appear to be declining, there does not appear to a genetic impediment to the recovery of AB pod.

The situation with the AT1 transient group is far more problematic. This group is clearly of the transient form based on both mitochondrial and microsatellite DNA. However, it can be seen from both Table 4 and Figure 2 that the transients of Prince William Sound are strongly subdivided, indicating that mating does not occur across groups. Indeed the AT1's are much more isolated genetically from the Gulf of Alaska transients than the Gulf of Alaska transients are from the transients of British Columbia. This is in keeping with our long-standing observation that AT1 and Gulf of Alaska transients do not associate. In view of its small size (approximately 11 individuals), it appears very unlikely that the AT1's can recover, due to either the genetic effects of inbreeding, or behavioural constraints against mating with close kin. The only reasonable possibility for recovery of the AT1's is by mating with another group. While we know of no related group of transients, killer whales have been poorly studied in the area south and west of Prince William Sound, and it is possible that a population exists in that area with which the AT1 transients could mate.

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 biphynel (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 12,13).

Table 12. Levels of total PCBs in selected groupings of killer whales (ppm, lipid weight).

	Resident Repro. Females	Resident Males	Resident First Born	Resident Non-First Born	Resident AB pod	Resident AK pod	All Residents	All Transients
n =	19	22	21	23	10	7	52	10
Mean	3.4	18.1	28.2	12.0	10.4	23.5	14.2	237.7
Stan. Dev.	1.6	12.2	16.8	5.7	5.7	17.8	14.1	136.1
C.I. (0.05)	0.8	4.7	7.2	2.4	3.6	11.6	3.4	84.3

Table 13. Levels of total DDTs in selected groupings of killer whales. (ppm lipid wt.)

	Resident Repro. Females	Resident Males	Resident First Born	Resident Non-First Born	Resident AB pod	Resident AK pod	All Residents	All Transients
n =	16	26	21	23	10	7	66	10
Mean	2.3	20.9	30.2	10.8	10.1	19.6	14.4	346.0
Stan. Dev.	1.7	20.1	21.6	5.3	5.4	15.3	16.8	224.5
C.I. (0.05)	0.8	7.7	9.3	2.2	3.3	10.0	4.5	139.1

northward in weather systems and condense and fall with rain in cooler northern regions (Iwata et al . 1993, Iwata et al. 1994, Tanabe et al. 1994). The contaminants are not excreted but stored in the lipids and bioaccumulate in consumers, increasing in concentration as they move up the food chain. Additional contaminants may have entered the aquatic environment from military dump sites in the Gulf of Alaska, although we have no idea of the kinds and amounts of materials that were disposed . This source is of great concern in the Aleutian Island area where sea otters were found to have substantial contaminant loads (reference).

Although there is the possibility of biological effects from contaminants even at low levels, we are particularly concerned about the relatively high levels of contaminants found in the transient whales. Long-term toxicity of environmental contaminants are expressed through carcinogenicity, teratogenicity, immunologic dysfunction and reproductive abnormalities(Tanabe et al. 1994). Of particular concern are the possibility that contaminant effects may be contributing to the lack of recruitment in the declining AT1 transient group.

The most thorough studies of contaminant effects have been conducted on laboratory animals. In rhesus monkeys there was embryonic resorption, abortion, still birth and irregular menstrual cycles at PCB concentrations of 71ppm /wet wt. of adipose tissue (Barsotti et al. 1976). In mice, Orberg and Kihlstrom (1973) found a prolonged oestrous cycle and a decline in the number of implanted ova with PCB levels between 44 and 424 ppm /wet wt in adipose tissue. In studies of free ranging pinnipeds, Helle et al. (1976) found that that the unusually numerous non-pregnant female seals had higher blubber PCB concentrations (77 ppm/wet wt.) than pregnant seals (56ppm/ wet wt.). Delong et al. (1973) found higher mean PCB and DDT concentrations in blubber of California seal lions aborting their fetuses (112.4 ppm/wet wt.) than in animals normally pregnant (17.1 ppm wet wt.). Dall's porpoise in the North Pacific revealed negative correlations between the residue levels of PCBs and DDE in the blubber and testosterone levels in the blood (Subramanian et al. 1987). Beluga whales in the St. Lawrence river estuary with PCB levels between 5.7 and 576 ppm/ wet wt. exhibited a failure to recruit offspring despite full protection from hunting after 1979. Martineau et al (1987) suggested that organochlorine contamination was a major factor in the non-recovery of this beluga population. The extremely high PCB contaminant levels (mean 393 ppm wet/wt.) found in striped dolphins in the Mediterranean Sea were linked to immune dysfunction (Kannan et al.1993) which led to a morbilavirus epizootic and a mass mortality of the dolphins.

We have graphically compared mean contaminant levels between resident and AT1 transient and GOA transient killer whales in our study with the mean contaminant levels measured for St. Lawrence Estuary beluga and for the Mediterranean striped dolphins (Figure 10). However, the lower average lipid content of Alaskan samples (27% lipid) versus the striped dolphin samples (41% lipid) and the beluga samples (91% lipid) underepresents the contaminant loads in Alaskan whales when compared by wet weight of sample. Although the Alaskan killer whale samples can be adjusted to determine contaminants based on lipid weight (as is reported for our data in Tables 12 and 13) those from the striped dolphins and beluga could not be adjusted because of variation in analytical techniques used in determining percent lipids. Thus, the true amounts of contaminants in the blubber of killer whales in our study is underepresented by an unknown factor in the wet weight comparison (Figure 10). We are currently working on methods of adjusting analytical results in other studies so that direct comparison by lipid weights can be made. The contaminant levels in the Alaskan whales are at least comparable to levels found in St. Lawrence Estuary beluga population where a lack of successful recruitment has been attributed to high contaminant loads (Martineau et al 1987).

ACOUSTIC ANALYSIS

Introduction

In the previous annual report (Matkin et al. 1998), we reported that resident killer whales in the Prince William Sound/ Kenai Fjords area used discrete calls in a manner similar to resident killer whales in British Columbia and Washington State (Ford 1989). Pod specific dialects composed of discrete calls can be used to identify pod membership of individuals. Discrete calls are heard in all behavioral contexts, however, they are used with greater frequency when whales are spread out foraging or when they are socializing with members of other pods. Therefore, it has been suggested that these pod specific dialects have dual functions (Tyack 1997). First, they appear to be used during cooperative feeding bouts on fish. Widely spread animals might signal to other pod members the forager's identity, state of arousal, and possibly prey abundance by means of a bistatic sonar (Tyack, 1997). Secondly, pod specific dialects are used in social gatherings of pods and could be associated with assortative mating between pods (Ford 1991). Dialect use in assortative mating has been considered a mechanism to avoid inbreeding (Treisman 1978). Further evidence for this second role has been suggested by Barrett-Lennard (pers. comm.) who found a positive correlation between nuclear DNA relationships and acoustic similarities in pods of resident killer whales in British Columbia. This second function is apparently responsible for the long-term stability of call structure, and makes our method of monitoring whale movements using their pod specific dialects possible.

In our last report we presented the pod specific call repertoires of six pods, AB, AD (now AD5 and AD16), AE, AI, AK, AN (now AN10 and AN20). Comparisons of pod specific dialects demonstrated a clear distinction between two pod clusters in the Prince William Sound/Kenai fjord resident population. The AB, AI, and AN pod cluster does not share calls with the AD, AE, and AK pod cluster. We now demonstrate that these clusters can be considered distinct clans, in which acoustic non-similarity indices correlate with the genetic distance between clans based on mitochondrial haplotypes. (Jurk et al. 1998, Matkin et al. 1998). The repertoire of AJ pod has also been analyzed and the pod placed in the appropriate acoustic clan.

In our previous annual report we analyzed recordings from a remote listening station in Prince William Sound and were able to identify the pods that were present on two occasions in January and February 1997. Results of the analysis of recordings made in Prince William Sound during the winter of 1997/98 are presented here.

Methods

Analytical techniques follow Matkin et al (1998) and are similar to those developed by Ford (1984) to analyze calls from resident killer whales in British Columbia. It has also been applied successfully to vocalizations of resident-type killer whales in Norway (Strager 1995), and to vocalizations of the isolated AT1 transient group of killer whales in Prince William Sound, Alaska (Saulitis 1993). The procedure combines a qualitative structural analysis of call types and call-type variants with a quantitative call-type frequency index to assess repertoires and repertoire sharing (Matkin et al. 1998).

The values Table 15 were used as a reference for the analysis of frequency of call occurrence in the 1997/98 recordings from a remote listening station at Bishop Rock near Montague Strait in Prince William Sound.

Call repertoire of AJ pod and its acoustic relationship to the other resident killer whale pods.

Recordings of AJ pod have been made during a total of 31 encounters between 1984 and 1996. The vocalizations of AJ pod are fall into the same overall categories typical for all killer whales. Aside from clicks and whistles, AJ pod uses an average of 9 discrete calls. Two of these calls, call-type AKS 10 and AKS 22[1], are shared call-types with AB, AI, and AN pod, and two sub-types, AKS 11iii and AKS 11iv, are new variants of a call-type used by the same group of pods. The other 5 call-types, AKS 23, 24i, 24ii, 29i, and 30, are unique calls of AJ pod. Therefore, AJ pod can be considered a member of the cluster previously described as comprising of AB, AI, and AN pod (Matkin et al. 1998).

In light of these results, we calculated the degree of vocal relatedness between each of the pods of each of the two clusters using the acoustic similarity index. The results of this analysis is presented in Table 16.

AB	1.0						
AD	0	1.0					
AE	0	0.533	1.0				
AI	0.889	0	0	1.0			
AJ	0.167	0	0	0.095	1.0		
AK	0	0.8	0.533	0	0	1.0	
AN	0.696	0	0	0.6	0.118	0	1.0
	AB	**AD**	**AE**	**AI**	**AJ**	**AK**	**AN**

Table 16: Acoustic similarity between pod repertoires calculated as a value between 0 and 1, where 1 means the repertoire of two pods are identical and 0 means the two pods do not share any call.

Using the values of Table 16 we designed a dendrogram to visualize the vocal relatedness. The dendrogram is based on a single link cluster analysis of the association between pods. The dendrogram is shown in Figure 11.

Using pod specific calls and differences in call-type frequency between pods according to Table 15 to determine the probability of the presence of a particular pod indicates that AK was the only pod present on October 23 and December 04, 1997. On December 12, 1997 there were AB and AJ pods present while on March 01, 1998 AD and either AF or AG pods were present.

Discussion

Ford (1991) suggested that acoustic relationships or vocal relatedness reflects the historic relationships between pods rather than the current ones, which are reflected by traveling associations. He proposed that vocal relatedness is an indicator for ancestral or genetic relatedness of pods. According to this AB, AI, AJ, and AN pod should have had a common ancestor as should AD, AE, and AK pod. The two groups of pods could therefore be considered two vocal clans (AB-clan and AD-clan).

Using DNA from biopsy samples, Barrett-Lennard sequenced the entire mitochondrial D-loop of individuals from each pod in AB clan and AD clan (see genetics section, this report). All of the AB-clan pods were monomorphic for one haplotype, and all of the AD-clan pods were monomorphic for a second haplotype. It thus appears that the acoustic differences between the clans, which we presume to be cultural, reflect differences in maternal ancestry. Therefore, it is appropriate to use call repertoires as a means of identifying pods over a very long period of time. Furthermore, we can use call sharing as an indicator of relatedness. This has important applications in the discovery of new pods or groups as an aide in determining whether new whales are related to members of the Prince William/Kenai Fjord community.

the Prince William Sound/Kenai Fjords resident population, we found the presence of two resident haplotypes that are identical to the northern and southern resident haplotypes from British Columbia and Washington State. Individuals within pods are consistently a single mtDNA haplotype, although resident pods of different haplotypes swim together. This separation is supported by the by vocal repertoire analysis (see below).

Nuclear DNA analysis determined eleven microsatellite loci that could be used in our analysis. Results confirmed the basic population separations established by MtDNA analysis. However, both mtDNA determined resident clans in Prince William Sound/Kenai Fjords were found to be closely related to the northern resident population of British Columbia. This indicates either direct outbreeding with that group, or more likely, transfer of genetic material via southeastern Alaskan resident pods. The southeastern Alaskan AG and AF pods also travel to Prince William Sound /Kenai Fjords and have been observed in association with northern residents. This again suggests the importance as potential breeding aggregations such as the multi-pod aggregations that occur in August/September in our area. In addition, microsatellite analysis has determined that resident whales seldom, if ever, breed within their pods of origin; in fact, they appear to select mates that are least closely related to them. Resident killer whales are not inbreed in respect to their subpopulations. We suspect resident killer whales use vocal dialect cues to determine relatedness of potential mates.

Unexpectedly, we found a group of whales with a new transient haplotype in Prince William Sound in 1998. Although very similar to the Gulf of Alaska haplotype, it was consistently different for the two animals sampled. Their relationship to the proposed Gulf of Alaska transient population is not clear.

The AT1 transients were found to be genetically isolated following results of nuclear and mtDNA analysis. The microsatellite analysis confirmed the substantial genetic distance between the AT1 whales, the British Columbia/southeastern Alaska transients, the proposed Gulf of Alaska transients as well as the new transient haplotype discovered in 1998. Based on this analysis and the association patterns we have observed over 15 years, it is doubtful that the AT1 group will interbreed with these other known transient populations. Since there are few animals remaining (11 total) and their are possible genetic effects or behavioral constraints on inbreeding, the AT1s may be unable to recover unless a genetically similar population exists elsewhere and becomes available for interbreeding.

Knowledge of sex ratios of all age classes is required for the construction of accurate life tables. We have continued the use of genetic sexing techniques to determine gender of immature individuals that have not been sexed by visual inspection of the genital area in the field.

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 are 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. Contaminant levels in transient killer whales were comparable to those found in Gulf of St. Lawrence beluga whales. In this beluga population, contaminants have been implicated in the non- recovery of the population from severe hunting as evidenced by lack of recruitment. We are concerned that contaminants may impede recruitment within the AT1 transient group if suitable mating opportunities exist.

Acoustic recordings made during 1984 to 1996 clearly separated resident and transient whales and demonstrated that resident pods have distinct pod specific repertoires. The genetic division of Prince William Sound residents is congruent with clearly discernible differences in vocal call repertoires, suggesting a long-standing cultural separation along matrilines. Resident pods have been separated acoustically into the same

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