Exxon Valdez Oil Spill
Long-Term Monitoring Program (Gulf Watch Alaska) Final Report

Long-Term Killer Whale Monitoring in Prince William Sound/Kenai Fjords

Exxon Valdez Oil Spill Trustee Council Project 16120114-M

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Study History: The North Gulf Oceanic Society independently maintained a monitoring program for killer whales (*Orcinus orca*) in Prince William Sound from 1984-1988 (Matkin et. al. 1994). This work was partially funded by a variety of non-profit foundations and government grants. Following the *Exxon Valdez* Oil Spill killer whales were monitored in Prince William Sound, Alaska with funding from the *Exxon Valdez* Oil Spill Trustee Council in 1989, 1990, and 1991 (Dahlheim and Matkin 1993) and in 1993 (Dahlheim 1994). The North Gulf Oceanic Society independently maintained a monitoring program in 1994. An assessment of the status of killer whales from 1984 to 1992 in Prince William Sound was published by Matkin et al. in 1994.

The current study builds upon this historical work as well as four other *Exxon Valdez* Oil Spill Trustee Council-supported projects, initiated in 1995 as Restoration Project 95012 "Comprehensive Killer Whale Investigations" and followed by "Photographic and Acoustic Monitoring of Killer Whales" initiated in 1999 and completed in 2002. The combined final report for these and later projects are available from the Alaska Resources Library and Information Services or from the North Gulf Oceanic Society as:

Matkin, C. O., G. Ellis, L. Barrett Lennard, H. Yurk, E. Saulitis, D. Scheel, P. Olesiuk, G. Ylitalo. 2003. Photographic and acoustic monitoring of killer whales in Prince William Sound and Kenai Fjords. *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project 030012), North Gulf Oceanic Society, Homer, Alaska.

Matkin, C. O., G. Ellis, E. Saulitis, D. Herman, R. Andrews, A. Gaylord, and H. Yurk. *In Review*. Monitoring, tagging, remote acoustics, feeding habits, and restoration of killer whales in Prince William Sound/Kenai Fjords 2003-2009. *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project 090742). North Gulf Oceanic Society, Homer, Alaska 99603

Matkin, C. O., G. Ellis, E. Saulitis, D. Herman, R. Andrews, and A. Gaylord. *In Review*. Monitoring, tagging, feeding habits, and restoration of killer whales in Prince William Sound/Kenai Fjords 2010-2012. *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project 10100742). North Gulf Oceanic Society, Homer, Alaska 99603.

Matkin, C. O., G. W. Testa, G. M. Ellis, and E. L. Saulitis. 2014. Life history and population dynamics of southern Alaska resident killer whales (*Orcinus orca*). Marine Mammal Science 30:460-479

Fernback, H., J. W. Durban, D. K. Ellifrit, J. M. Waite, C. O. Matkin, et al. 2014 Spatial and social connectivity of fish-eating resident killer whales (*Orcinus orca*) in the North Pacific. Marine Biology 161:459-472.

Bodkin, J. L., D. Esler, S. D. Rice, C. O. Matkin, and B. E. Ballachey. 2014. The effects of spilled oil on coastal ecosystems: lessons from the *Exxon Valdez* spill. Pages 311-346 *in* B. Maslo and J. L. Lockwood, editors. Coastal Conservation. Cambridge University Press, New York, USA.

Teerlink, S. F, O. von Ziegesar, J. M. Straley, T. J. Quinn II, C. O. Matkin and E. L. Saulitis. 2015. First time series of estimated humpback whale (*Megaptera novaeangliae*) abundance in Prince William Sound. Environmental and Ecological Statistics 22:345-368. DOI 10.1007/s10651-014-0301-8

Saulitis, E, L. A Holmes, C. Matkin, K. Wynn, D. Ellifrit, and C. St. Amand. 2015. Bigg's killer whale (*Orcinus Orca*) predation on subadult humpback whales (*Megaptera novaeangliae*) in Lower Cook Inlet and Kodiak, Alaska. Aquatic Mammals 41:341-344. DOI 10.1578/AM.41.3.2015.341

Filatova, O. A., F. I. P. Samarra, L. G. Barrett-Lennard, P. O. Miller, J. K. B. Ford, H. Yurk, C. O. Matkin, E. Hoyt. 2016. Physical constraints of cultural evolution of dialects in killer whales. Journal of the Acoustical Society of America 140:3755-3764.

Data from this project is also published in Olsen et al. (2018).

Abstract: In the four-year period, 2013-2015 a total of 249 days were spent in surveys with 138 encounters with killer whales (*Orcinus orca*). The *Exxon Valdez* oil spill-damaged AB pod contained 21 whales in 2015 and hadn't recovered to the pre-spill number of 27 individuals. The threatened AT1 transient population numbered 7 whales in 2015 and totaled 22 prior to the spill. Extinction is likely with no new calves produced since 1984. The 33 tags attached to resident killer whales indicated distinct shifts in core use areas that were highly specific to season and pod. Genetic analysis supported the area south of Kodiak Island as the southwestern limit of the Southern Alaska resident population as well as a boundary for the Gulf of Alaska transient population. Sampling of fish scales from southern Alaska resident predation sites indicates a pattern of Chinook salmon (*Oncorhynchus tshawytscha*) predation in the spring followed by increasing predation on chum salmon (*Oncorhynchus keta*) and Coho salmon (*Oncorhynchus kisutch*) the summer and fall. There has also been a decline in the average annual stable isotope levels in resident killer whales over the past twelve years. This suggests a change in food habits; possibly a decline in Chinook salmon and an increase in chum salmon in the diet.

Key words: feeding habits, foraging, genetics, Kenai Fjords, killer whales, offshore, *Orcinus orca*, photo-identification, populations, Prince William Sound, resident, transient

Project Data:

Data description and format - Data includes frame-by-frame analysis of photo-identification pictures and an annual tabulation of all whales present (Excel spreadsheet). In Excel spreadsheets all biopsy samples are described and results from chemical analysis are detailed. Catalogues of all known individuals and pods are provided in Powerpoint diagrams including most recent ID photo. Tracks and associated data for all tagged killer

whales are also provided. Long term summaries of field surveys and encounters are found in an ACCESS database.

Data location and access limitations - All data are available online at: http://portal.aoos.org/gulf-of-alaska.php#metadata/2f42dd1c-d67a-4c49-8c2e-1d63387e0ad0/project. All data are also archived by North Gulf Oceanic Society, 3430 Main St Ste B1, Homer, AK 99603. There are no limitations on the use of the data, however, it is requested that the authors be cited for any subsequent publications that reference this dataset. It is strongly recommended that careful attention be paid to the contents of the metadata file associated with these data to evaluate data set limitations or intended use.

Data contact – Carol Janzen, 1007 W. 3rd Ave. # 100, Anchorage, AK 99501, 907-644-6703, janzen@aoos.org, http://portal.aoos.org/gulf-of-alaska.php

Citation:

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EXECUTIVE SUMMARY

This report covers the four-year period from 2013-2016 of a 28 year photo-identification based study of killer whales (*Orcinus orca*) in Prince William Sound and Kenai Fjords, Alaska. It has followed four separate killer whale populations, the largest being the southern Alaska residents, but also the threatened AT1 (Chugach) transients, the lesser-known Gulf of Alaska transients and the infrequently seen offshore killer whales. In the current study period, 249 days were spent in surveys that covered 10,031 km between May 1 and October 30 of each year. There were 138 encounters with killer whales that spanned 1848 km. The *Exxon Valdez* oil spill-damaged AB pod contained 21 whales in 2015 and has not recovered to pre-spill number of 27 individuals. The threatened AT1(Chugach) transient population numbered 7 whales in 2015 and totaled 22 prior to the spill. The AT1(Chugach)transient population is likely headed toward extinction with no new calves produced since 1984. In 2015, there were 272 resident killer whales in the 10 frequently encountered resident pods used in population analysis. Based on photographs taken since 2004, we estimate a minimum population size for southern Alaska resident killer whales of 1062, of which 347 have not been assigned to pods.

We tagged 33 resident killer whales representing 14 pods in the southern Alaska resident population between 2006 and 2014 during the months of June to January. Distinct shifts in core use areas were revealed that are highly specific to season and pod. In June, July, and August, the waters of Hinchinbrook Entrance and west of Kayak Island were the primary areas used, mainly by the AB, AI, and AJ pods. These same pods shifted their focus to Montague Strait in August, September, and October. Port Gravina was a focal area for the AD16 and AK pods in June, July, and August, but this was not the case in later months. AK and AD16 pods were responsible for seven of eight documented trips by tagged whales into the deeper fjords of Prince William Sound. However, these fjords were not a focus for most groups. These temporal shifts in habitat use are likely a response to the seasonal returns of salmon.

In our genetic analysis we used data from 26 nuclear microsatellite loci and mitochondrial DNA sequences (988 bp) to test *a priori* hypotheses about population subdivisions. Our samples were grouped with other samples generated from a decade of killer whale surveys across the northern North Pacific. This work confirmed that the area south of Kodiak Island defines the southwestern limit of the Southern Alaska resident population as well as a similar southwestern boundary for the Gulf of Alaska transient population, which uses the ocean entrances of Prince William Sound and the Kenai Fjords region.

Sampling of fish scales from southern Alaska resident predation sites indicates an annual pattern of Chinook salmon (*Oncorhynchus tshawytscha*) predation in the spring followed by increasing predation on chum salmon (*Oncorhynchus keta*) and Coho salmon (*Oncorhynchus kisutch*) as the summer and fall progress. This is reflected also in the stable isotope levels in the blubber of the killer whales over the course of the season. Additionally, the average annual stable isotope levels of the whales have declined over the past 12 years. This change has not been observed in the Southern residents of Puget Sound region. This indicates either a change in stable isotope values across the entire Gulf of Alaska ecosystem

or a change in feeding habits over the past 12 years. Considering there is little evidence for a trophic ecosystem shift of that magnitude, it is more likely that southern Alaska resident feeding habits have changed which is supported by increased chum salmon samples from predation sites. Further evidence of this shift in diet is provided by the surprisingly strong annual decline in DDT levels in southern Alaska residents (8%) while the Southern Residents of Puget Sound have only had DDT declines of about 2% per year. The apparent change in prey composition may be due to the decline of abundance of Chinook in the region over the past decade or due to the expanded nutritional needs of an increasing population of resident killer whales (excluding the oil spill-impacted AB pod).

It appears AT1 killer whales remain primarily harbor seal (*Phoca vitulina*) predators with Dall's porpoise (*Phocoenoides dalli*) also an important component of the diet. Gulf of Alaska transients appear to focus on Steller sea lions (*Eumetopias jubatus*) and recently we have observed an increasing number of predation events on Dall's porpoise by whales from this population. Offshore killer whale prey samples continue to indicate Pacific sleeper shark (*Somniosus pacificus*) as the primary prey in the northern Gulf of Alaska in summer.

INTRODUCTION

Population monitoring of killer whales in Prince William Sound and adjacent waters has occurred annually since 1984. The existence of data prior to the *Exxon Valdez* oil spill made it possible to determine that the resident AB pod and the AT1 (Chugach) transient group declined dramatically following the spill (Matkin et al. 2008). This project continued using photo-identification methods to monitor changes in resident killer whale pods and monitor recovery of the AB pod and the AT1 transient population. We continued to emphasize photo-identification during the funding cycle 2013-2016 over all other aspects of the project. A journal paper detailing the population dynamics of resident killer whales in the northern Gulf of Alaska was published during this funding cycle (Matkin et al. 2014). In this report we update status of the AB pod and the AT1 transient population as well as regularly sighted groups and infrequently sighted whales to develop an overall population estimate and establish population trend.

Both whales of the resident ecotype, the AB pod, and whales of the transient ecotype, the AT1 population, suffered significant mortalities following the *Exxon Valdez* oil spill in 1989. The AB pod is recovering after 26 years but has still not reached pre-spill numbers. The AT1 population is not recovering and may be headed toward extinction. This project has determined that killer whales are sensitive to perturbations such as oil spills, but has not yet determined the ultimate long-term consequence (which may include extinction) or the recovery period required after such a perturbation. Ecosystem changes may also complicate aspects of recovery. As an apex predator, this species (both fish and mammal eating ecotypes) has an important role in the "top down" processes of the ecosystem. Additionally, they are a primary focus of viewing in the region by a vibrant tour boat industry. Data from this project is used by tour boats to enhance viewer's experience and to promote appreciation of the local environment and fauna. Unlike many cetaceans, killer whales can be closely monitored using photo-identification and other investigative tools. This long term monitoring project is a unique opportunity to continue a comprehensive

database initiated in the early 1980's for one of the regions keystone marine species. The importance of long-term killer whale monitoring has been borne out by companion studies in other regions such as Puget Sound and British Columbia.

In this project we used photo-identification, prey sampling, biopsy sampling, and satellite tagging to develop population parameters and to study feeding ecology, range, and distribution. Analyses include population dynamics, genetics, examination of lipids and fatty acids and environmental contaminants in the blubber, and development of location and dive data from satellite tags. Although we focused on the southern Alaska resident and AT1 transient populations, which were impacted by the spill, the study also included the other two recognized populations in the region, the Gulf of Alaska transients and offshore killer whales and contributed substantially to the National Marine Fisheries Service (NMFS) killer whale stock assessments.

Data were collected during a minimum 50-day field season from May through October from the R.V. *Natoa*. In addition, other collaborating vessels contributed opportunistic photographic data. This is the continuation of a long-term project initiated in 1984 and has benefited from continued support of the *Exxon Valdez* Oil Spill Trustee Council and from individuals living in coastal communities along the north Gulf coast of Alaska.

OBJECTIVES

1) Photo-identification of all major resident pods and AT1 (Chugach) and other transient groups that use Prince William Sound/Kenai Fjords on an annual basis. Realistically, all pods are completely documented on a biennial basis, despite annual field effort. Extension of individual histories, identification catalogues of individuals and an annual update of population were products of these data.

2) Collection of blubber samples for chemical monitoring of PCBs, DDTs and PBDEs, lipid and fatty acid (FA) content and stable isotope values to gauge changes in contaminant loads as examine feeding habits.

3) Collection of fish scale samples and marine mammal tissue from kill sites to monitor potential changes in feeding habits.

4) Collection of skin samples for genetic analysis.

5) Tracking of individuals from a variety of pods using ARGOS satellite telemetry to improve re-sighting rate in the field and foster completion of objectives 1-3.

6) Determine details of range of pods and populations using both ARGOS and photo-identification data and identify important habitat on a pod specific basis.

METHODS

The vessel surveys conducted in this project focused on the bays and passes of Prince William Sound and the Kenai Fjords region and particularly the ocean entrances (Fig. 1) These waters are glacially carved and relatively deep (300-500m), and experience strong

tidal currents (Halverson et al. 2013). Strong downwelling conditions in winter promote inflow into Prince William Sound through Hinchinbrook Entrance and outflow through Montague Strait, but this pattern is less distinct in the summer months as offshore downwelling conditions relax (Halverson et al. 2013).

Data Collection

Fieldwork during the 2013-2016 study period was completed from the R/V *Natoa,* a 10.3 m inboard diesel powered vessel, capable of 12 knots and sleeping four researchers. Data were recorded on daily vessel logs and killer whale encounter sheets (updated in 2014) and basic field data was input into an ACCESS database. Vessel tracks and encounter tracks were recorded on a Garmin Mark V GPS and converted to GIS shapefiles for analysis.

Researchers attempted to maximize the number of encounters with as many killer whale pods or groups as possible and based field timing and search tracks on current and historical sighting information. Consequently, searches were centered in areas that had produced the most encounters with killer whales in the past, unless sighting or report information indicated changes in whale distribution. Satellite data (Olsen et al. 2018), Appendix 1) supported long term survey areas as killer whale hot spots. Whales were found visually, by listening for killer whale calls with a directional hydrophone, or by responding to VHF radio calls from other vessel operators. Regular requests for recent killer whale sightings were made on hailing Channel 16 VHF. In Kenai Fjords, Channel 71, the tour boat channel was also monitored in Kenai Fjords. 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 or provided on our website by other mariners if of sufficient quality and accompanied by appropriate data. Photographs for individual identification were taken of the port side of each whale showing details of the dorsal fin and saddle patch. Digital images were taken at no less than 1/1000 sec. using a Nikon D-700 or D-750 camera and a 300mm f4.5 auto focus lens. 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 vessel operated using a Garmin GPS V that was downloaded each evening. Tracklines were then converted to GIS shapefiles using Minnesota DNR Garmin 5.4 software. Similar logs were kept for all previous study years and stored as shapefiles with encounter tracks separated from overall vessel tracks and used to estimate effort (Scheel et al. 2001). On daily logs, the elapsed time and distance traveled were independently recorded. Weather and sea state as it affected daily surveys was noted.

Specifics of each encounter with killer whales were recorded on standardized data forms originally developed in 1984. These forms have been updated every few years to reflect changes in data collection needs and emphasis (most recently modified in 2016). Data

recorded included date, time, duration, and location of the encounter. References to digital photographic files were created and the estimated number of whales photographed also were recorded. Specific group and individual behaviors (i.e., feeding, resting, traveling, socializing, milling) were recorded by time and location. Directed observations of feeding behavior and identification and collection of killer whale prey and fecal material were made when possible.

Evidence of resident killer whale predation was collected using an extendable, fine mesh, dip net to retrieve fish scales or pieces of flesh from prey at the site of a kill. This collection technique provided prey species identification as well as data on the life history of the prey as determined from scale annuli. Scales were aged and identified at the Pacific Biological Station, Nanaimo, British Columbia by making acetate impressions and viewing the impressions on a Neopromar projecting scope. Magnifications of 10x to100x were used in the analysis (MacLellan 2004). Sampling of prey was coupled with standard killer whale photo-identification procedures (detailed in Bigg et al. 1990 and Matkin et al. 1999) to determine the identity of the population, the pod, and, in some cases, the individual whale, using existing photographic catalogues (Matkin et al. 1999). Sampling of prey remains occurred opportunistically during the period of our annual photo-census (April-September). Time and location of all predation events were also recorded.

Foraging behavior by a group of resident (fish eating) killer whales was initially identified acoustically by the presence of echolocation clicks and discrete calls detected using an Offshore Acoustics omni-directional hydrophone (100Hz to 25 kHz). In addition, there were visual cues such as erratic movements of widely spaced individuals. As in our previous study (Saulitis et al. 2000), predation events accompanied by noticeable whale surface activity typically triggered our movement to the kill site and the attempted collection of scale samples. We also were successful in obtaining scale samples by following an individual (or cow/calf pairs) for extended periods during foraging and waiting for successful feeding to occur. However, the capture of prey at depth is not always accompanied by obvious surface activity, although the whale may occasionally carry prey to the surface. This made extended follows of individuals more productive at times than searching for obvious surface kills.

Killer whale feces were also collected in the last year of the study and genetic analysis completed at Northwest Fisheries Science Center (NWFSC), Seattle, Washington by Kim Parsons. Killer whale feces were collected with a fine mesh net on an extendible handle (4 m maximum extension). The net was rinsed thoroughly and sprayed with dilute chlorine solution between sampling to prevent genetic contamination. The pod or group of killer whales and specific individuals present being tracked when feces were collected were identified and recorded on the encounter data sheets.

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

When successful predation was suspected, the kill site was approached slowly. An observer on the bow of the research vessel scanned the area and retrieved fish scales or other prey fragments using a long handled dip-net. Samples were placed in envelopes labeled with the date, time, location of the kill site, and the identity and/or pod designation of the animal making the kill.

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

Biopsy samples were collected using a pneumatic rifle and custom-designed biopsy darts (Barrett-Lennard et. al. 1996). A small dart was fired from a specially outfitted rifle powered by air pressure from a .22 caliber blank cartridge. The setup is similar to that used to deliver tranquilizing drugs to terrestrial mammals in wildlife research. A lightweight plastic and aluminum dart (approximately 10 cm long by 1.2 cm diameter) was fitted with a beveled tubular sterile stainless steel tip that took a small core of skin and blubber (approximately 1.6 cm long and 0.5 cm diameter). The sterilized dart was fired from a range of 16-20 m. The dart struck the animal in the upper back, excised a small tissue sample, bounced clear of the whale, and floated with sample contained until retrieved with long handled net.

From the biopsy samples, the epidermis, which is heavily pigmented, was separated aseptically from the other layers with a scalpel soon after retrieval. The dermal sample used for genetics and stable isotope analysis, was stored at about 4° C in a sterile 1 ml cryovial. The dermis and hypodermis were made up primarily of collagen and lipid, respectively, and were frozen at -20° C in autoclaved, solvent-washed vials for contaminant analysis. Specifically, each biopsy sample was analyzed for their skin carbon and nitrogen stable isotope (SI) ratios, blubber fatty acids (FAs), and persistent organic pollutants (POPs). Lipid class analyses were also conducted on all blubber samples but those results will not be described here.

ARGOS m onitored, location only Spot 5 satellite tags or Mark10 time/depth/location tags produced by Wildlife Computers, Seattle, WA were attached to the dorsal fin of killer whales to track longer term movements, determine range and important habitat, map time and depth of dives to determine behaviors in particular locations. A small barbed dart protruding 5 cm into the dorsal fin of the adult male killer whale was implanted as part of the tag to anchor it in the connective tissue. Attachments were made from distances of approximately 8-15 m by crossbow using a Barnett Wildcat 170-pound bow or similar.

Acoustic recordings were made using an Offshore Acoustics omnidirectional hydrophone lowered over the side of the vessel in combination with Tascam professional digital recorder. Audio files in .wav file format were downloaded after each encounter. The hydrophone had a flat frequency response to signals ranging from 100 Hz to 25 kHz. The tape recorder showed a flat response to signals up to 15 kHz.

Photo-identification

To meet Objective 1, digital images were examined using PhotoMechanic software (CameraBits Inc.) on an Apple computer with a 24-inch high resolution LCD screen. Identifiable individuals in each image 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. (1990) and Heise et al. (1992) and has been continued in the catalogue of southern Alaska killer whales (Matkin et al. 1999). More recently we have posted an updated catalogue of individuals on our website (whalesalaska.org. 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 compiled on a frame-by-frame basis and individuals present in each encounter were tabulated and recorded in a continuing digital database. From this photographic database, the actual number of whales identified and pods of whales present for each encounter was determined and included with each encounter summary entered in the Database of Surveys and Encounters tabulating all surveys and resulting encounters. These and other data from this project found on AOOS Ocean Workspace:
https://workspace.aoos.org/group/4601/project/4682/folder/4879/data

Habitat Use

To meet Objectives 5 and 6 we conducted a satellite telemetry project which is described in detail in Appendix 1**:** Olsen et al. (2018).

Genetic Analysis

To meet Objective 4 we conducted a genetic study. See Appendix 2: Parsons et al. (2013).

Feeding Habits

To meet Objectives 2 and 3, dietary and behavioral data were gathered concurrently with census data during this study although of reduced priority during this current study period (2013-2016) in light of reduced field time. Although periods spent in the field varied among years, data collection occurred primarily May-September in all years (see above).

Laboratory analysis included measurements of skin carbon and nitrogen stable isotopes were conducted following the procedure described in Herman et al. (2005). In essence, the procedure involves freeze-drying ~50-200 mg of wet skin tissue, removing lipid by accelerated solvent extraction using methylene chloride, pulverizing the lipid-free skin to a powder in a micro ball mill, loading ~500ug of powder into tin cups and combusting the powder in a Costech elemental analyzer attached to a Thermo-Finnigan Delta Plus Isotope Ratio Mass Spectrometer. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios were measured relative to Vienna Pee Dee Belemnite and atmospheric nitrogen, respectively.

Blubber FAs were analyzed following the procedure described in Herman et al. (2005). Prior to analysis, all blubber biopsy samples were sub-sampled by performing two lateral cuts, the first ~1mm from the inside edge of the epidermis tissue and a second cut exactly 20 mm from the epidermis-blubber interface. Because FAs are highly stratified in killer whale blubber tissues (Krahn et al. 2004), it was necessary to standardize all blubber samples in this fashion in order to represent a constant blubber depth. These standardized blubber tissues were then extracted by ASE using methylene chloride, an aliquot containing approximately 2 mg total lipid (typically less than 4% of the total extract) and transesterified to the respective FA methyl esters (FAMEs) using 3% sulfuric acid in methanol. The FAMEs were then extracted into iso-octane, and these final extracts separated and analyzed on a 60m DB-23 capillary column using a quadrupole gas chromatography/mass spectrometer (GC/MS). All FAME concentration data are expressed on a weight-percent basis (wt %) by dividing the concentration of each individual FAME by the sum of all FAMEs present in the sample.

Blubber POPs were analyzed following the procedure described in detail in Sloan et al. (2005). In short, the method involves cleanup of half or more of the lipid extract described above for the analysis of FAs (which also contains POPs) on a silica/alumina column to remove polar extraneous compounds, separation of the POPs from all lipids by High Performance Size Exclusion Chromatography (HPSEC), and finally separation and analysis on a 60m DB-5 capillary GC column equipped with a quadrupole mass spectrometer operated in the selected ion mode. POP concentration data were lipid normalized and expressed in units of ng POP/g lipid. In contrast, PCB profile data are expressed on a wt % composition basis by dividing the lipid-normalized concentration of each individual PCB congener by the sum of the lipid-normalized concentrations of all congeners measured in the sample.

All multivariate and univariate analyses of the stable isotope and contaminant data obtained in this study were conducted using either JMP Statistical Discovery Software (PC professional edition version 5.01) or Primer-E Software (version 6.16). Unless indicated otherwise, all univariate comparisons between two groups were significance tested (α=0.05) using a simple two-sample Student's t-test assuming unequal variances. Significant differences among multiple groups assumed to have approximately equal variances were evaluate using a Tukey HSD test (α=0.05).

RESULTS AND DISCUSSION

Summary of Effort and Encounters

During the period of this study, 2013-16, the R/V *Natoa* spent a total of 249 days on the water searching for killer whales along 10,031 km of trackline for an average search distance of 40.3 km day. Killer whales were encountered on 138 occasions and followed over a distance of 1848 km, approximately 13.4 km per encounter (Tables 1, 2; Figs. 1, 2).

Table 1. Summary of effort tracking killer whales in Prince William Sound and Kenai Fjords, Alaska.

Year	# Vessel days	Distance Surveyed (km)
2013	53	2114
2014	70	2658
2015	65	2788
2016	61	2471
TOTAL	249	10,031

Table 2. Summary of encounters with killer whales in Prince William Sound and Kenai Fjords, Alaska.

Year	# Encounters	Distance traveled with whales (km)
2013	21	282
2014	36	585
2015	45	588
2016	36	392
TOTAL	138	1848

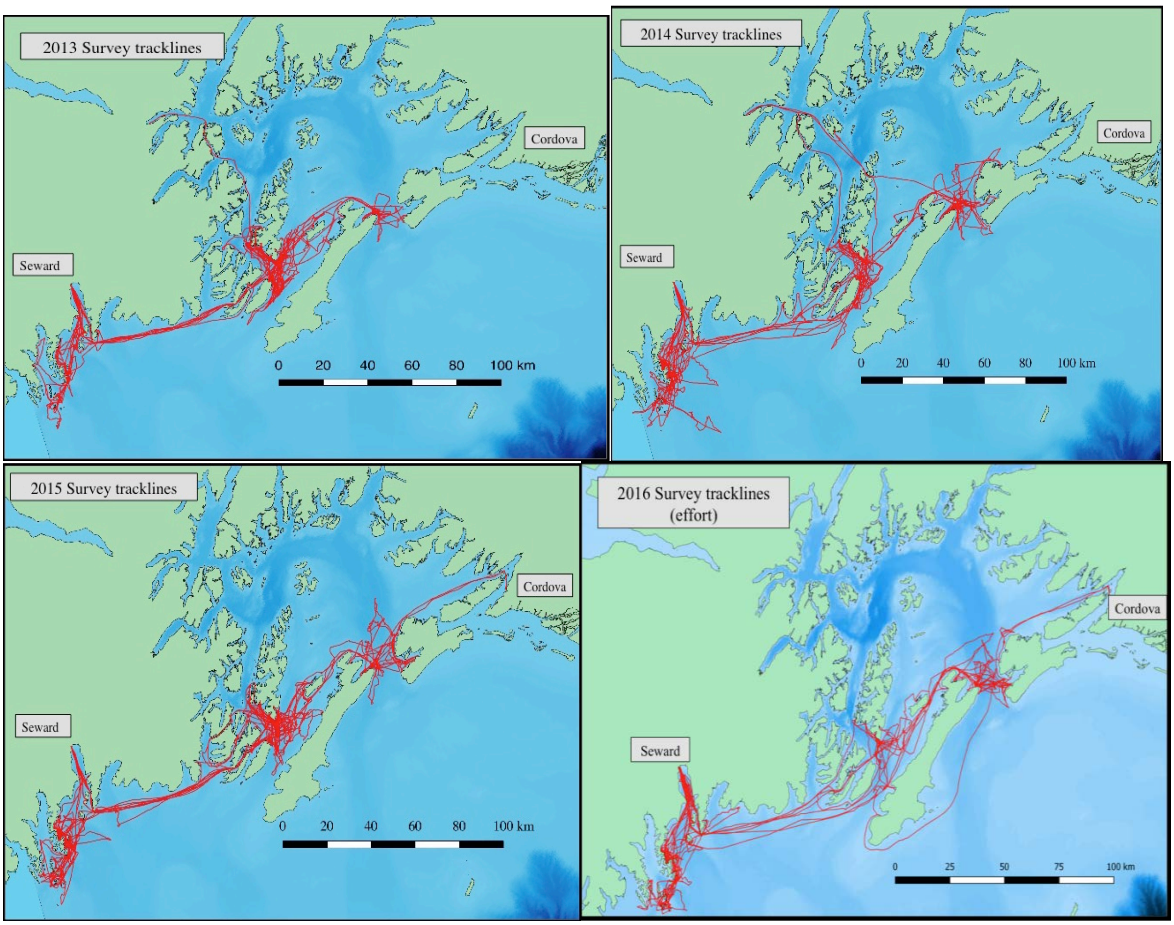

Figure 1. Killer whale survey tracklines of the vessel R/V *Natoa* in Prince William Sound and Kenai Fjords, Alaska, 2013-2016.

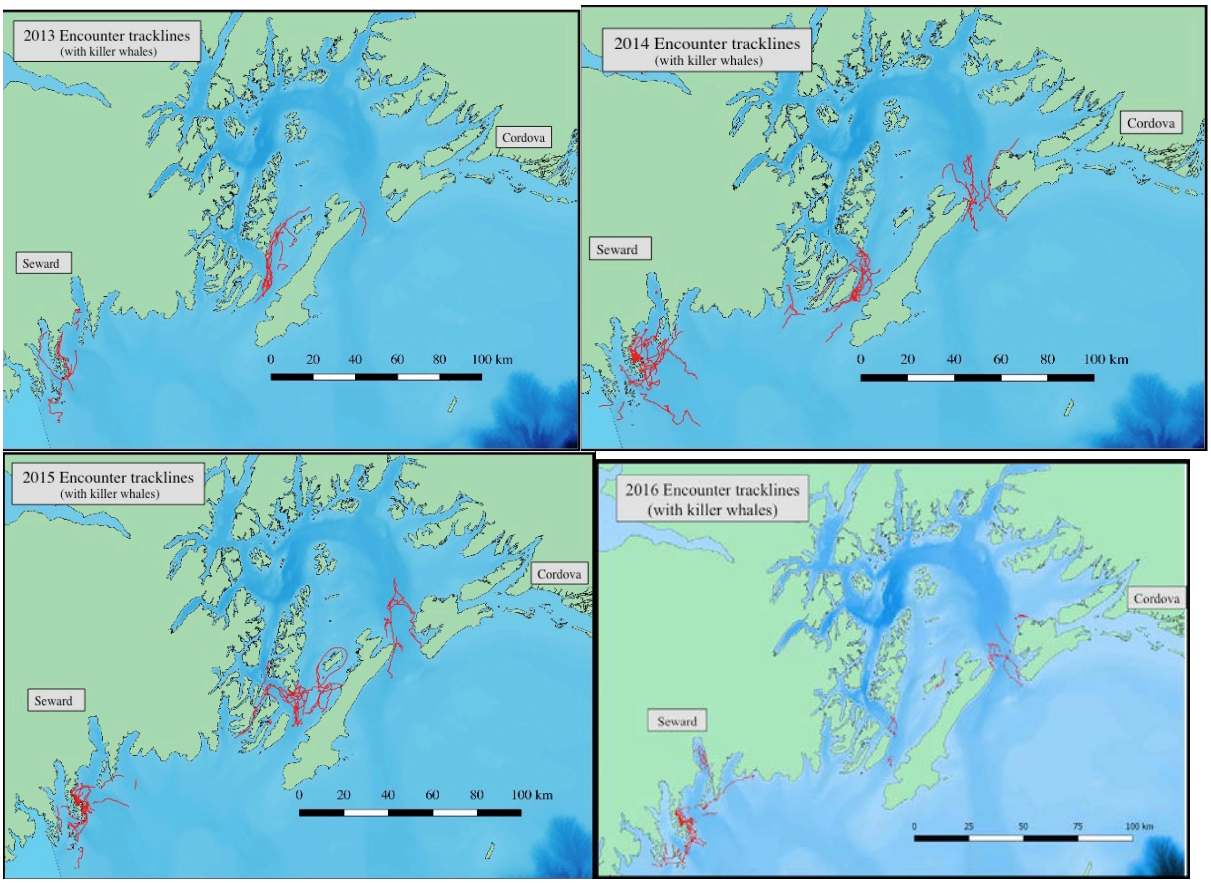

Figure 2. Tracklines of encounters with Killer whales from the R/V *Natoa* in Prince William Sound and Kenai Fjords, Alaska, 2013-2016.

Population Trends

Killer whales have a cosmopolitan distribution and are top-level predators. Our studies in the northern Gulf of Alaska, which were initiated in the mid-1980s indicate that at least three ecotypes exist in this region: residents, transients, and offshores (Matkin et al. 1999, Ford et al. 2000). Despite their sympatric distribution, these ecotypes do not associate or interbreed and are acoustically and genetically distinct. Our population studies have focused primarily on the AB pod, the southern Alaska resident population and the AT1 transient population. Both the AB pod and the AT1 transients lost individuals following the *Exxon Valdez* oil spill (Matkin et al. 2008) and neither have recovered to their pre-spill numbers.

Although there was a slight decline in the AB pod in years prior to the spill, the pod had increased to 27 whales in the fall prior to the spill (Fig. 3). Shortly after the oil spill, apparently due to social changes within the pod following oil spill-related mortalities, part of the AB pod split off (AB25 pod). The remaining core of AB pod (which numbered 27 whales pre-spill) has been slowly recovering since the spill. In the current study, the AB pod was last photographed completely in 2015 and only partially photographed in the final

year of work in 2016. There were no new deaths and two new calves were recruited in 2015 and the pod now totals 21 individuals.

In 2015 for the first time in several years, we were able to photo-document all of the remaining seven individuals of the AT1 (Chugach) transients in a single year. It is often difficult to locate AT6, an elusive male that often travels alone and these whales appear to have shifted their historical range to spend more time in glacial areas where we seldom operate (Fig. 4). Complete coverage was possible because of cooperative effort with other mariners, particularly tour boats that travel to tidewater glacial areas (e.g., Columbia Glacier, Holgate Glacier).

During the period ending 2015 (the 2016 data could not be used until mortalities are confirmed in 2017 whales must be missing for two years to be considered dead). We documented 272 resident killer whales in the 10 frequently encountered resident pods (AB pod excluded; Table 3) which represents a substantial increase in southern Alaska resident numbers from the 197 whales reported in 2010. These pods have been used in tracking population trends and in examining population dynamics since 1984 (Matkin et al. 2014). There appears to be little slowing in birth rate nor is there a rising mortality rate as we would expect as the population approaches carrying capacity. Although the population has been growing for the more than 30 years we have been tracking them, there seems to be no slowing of this growth averaging slightly over 3% per year and may be representative of a population at rmax (Matkin et al. 2014). Eventually southern Alaska residents would be expected to reach equilibrium with carrying capacity and end this period of growth. Due to our study design, this slowing or decline should be clearly observable when it occurs. As these resident (fish eating) pods continue to grow, there has been some splitting of pods and some changes in range (Olsen et al. 2018, Appendix 1). The growth seems still to be driven by the long-term rebound of salmon from depleted levels in the mid-1900s (Matkin et al. 2014; see Feeding Habits, this report).

The minimum number of whales in the southern Alaska resident population we now place at 1062 (Table 4). This includes all the whales we have photographed since 2004 across their range from southeastern Alaska through Kodiak. It includes 347 whales that have not been assigned to pods. Although some of these whales may have died since they were last photographed, it is unlikely that we have photographed all pods and individuals in the population and consider this a minimum population estimate.

We continued to get reasonable coverage of transient killer whales (both AT1 and GAT populations) in most years despite the reduction in field time from peak years of the study following the oil spill (Table 5, Fig. 4). In part, this was accomplished by using contributed photographs from vessels of opportunity. We have no current minimum estimate or trend data for the Gulf of Alaska transients at this time but there has been recruitment observed in some more frequently observed groups that suggest there has not been a decline since our last population analysis (Matkin et al. 2012).

We encountered the shark-eating offshore ecotype killer whales on only four occasions during this study period (Table 6). Encounters with offshore killer whales have always been infrequent and unpredictable in the study area. There is not a minimum population

estimate across their range, which extends from Alaska to California; however, there are over 400 whales in the offshore identification catalogue maintained at the Department of Fisheries and Oceans Canada, Nanaimo, British Columbia, Canada.

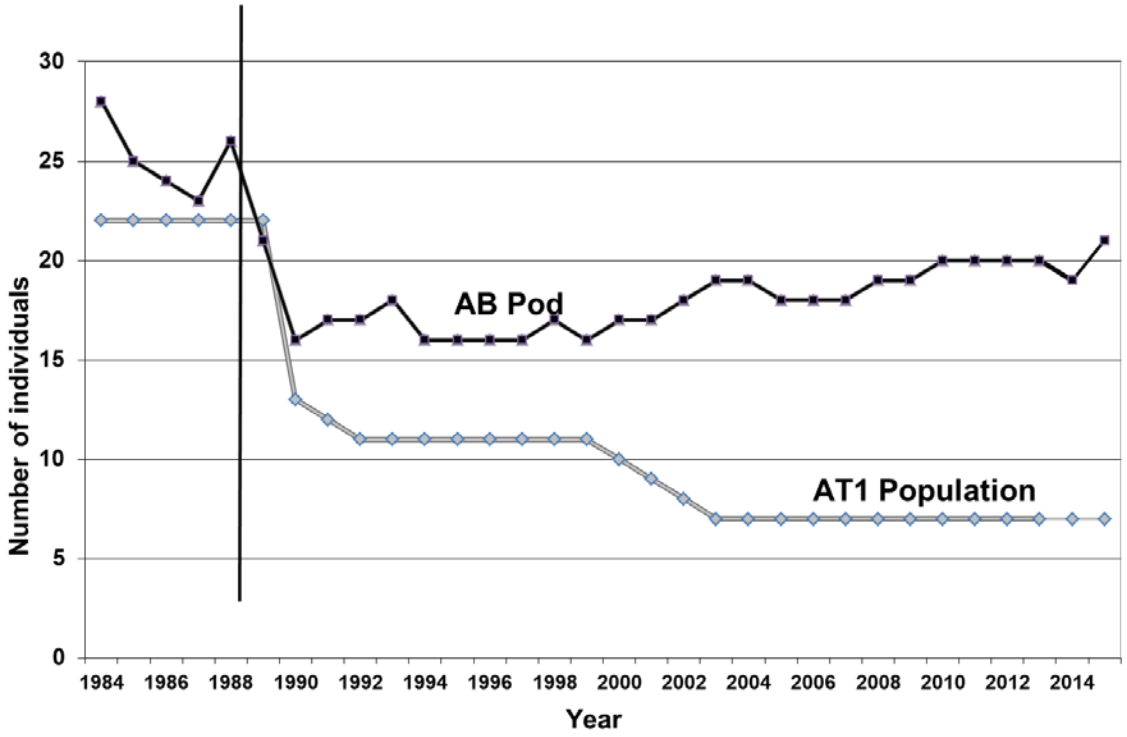

Figure 3. Number of killer whales in the AB pod and AT1 population from 1984 to 2015.

Table 3. Recruitment, mortalities, and total number of killer whales since 2010 (unless otherwise noted) for frequently seen resident pods.

POD	Total 2010	Total Recruited since 2010	Total Died since 2010	Year of last census	Most Recent Total
AB25	18	7	1	2015	24
AD05	19	7	0	2015	26
AD16	8	3	1	2015	10
AE	17	4	3	2015	19
AG	39 (2005)	13	2	2015	50
AI	7	4	2	2015	9
AJ	55	17	5	2015	67
AK02	9	6	1	2015	15*
AK06	6	4	1	2015	8**
AY	19	6	2	2015	23
TOTAL	**197**			**2015**	**272**
AB	20	6	5	2015	21

*one animal gained by immigration from AK06 pod
**one animal lost through immigration to AK02 pod

Table 4. Estimated total population of southern Alaska resident killer whales in 2015 by pod and including animals not assigned to pods. Regularly monitored resident pods are in bold.

Pod (may be a single matriline)	**Number of whales**	**Year last completely documented**
AA1 and AA30	32	2010
AB	**21**	**2015**
AB25	**24**	**2015**
AD05	**26**	**2015**
AD16	**10**	**2015**
AE	**19**	**2015**
AF5	**46**	**2010**
AF22 (not incl AF16s)	**31**	**2014**
AG	**50**	**2014**
AH01 and AH10	21	2010
AI	**9**	**2015**
AJ	**67**	**2015**
AK	**23**	**2015**
AL	23	2010
AM	7	2014
AP	19	2012
AN10	**36**	**2012**
AN20	30	AN29s in 2007, AN15s AN69s and AN 32s in 2005 and AN23s in 2002
AS2	32	2012
AS30	19	2012
AW	27+	2010
AX01	29	2008
AX27	26	2010
AX32	21	2010
AX40	16	2010
AX48 (not including AX48, AX50, AX55 and AX56)	28	2015
AY	**23**	**2015**
Unassigned to pods	347 (crude count)	Seen between 2004 and 2015
TOTAL SEA to KODIAK	**1,062**	

Table 5. Sighting histories for all AT1 transient whales for years with effort greater than 40 days. “X” indicates the animal was present, “O” indicates missing and a carcass was found, and “-” indicates missing and presumed dead.

	AT1	**AT2**	**AT3**	**AT4**	*AT5*	**AT6**	*AT7*	*AT8*	**AT9**	**AT10**	*AT11*	*AT12*	**AT13**	**AT14**	*AT15*	*AT16*	**AT17**	**AT18**	*AT19*	*AT20*	*AT21*	*AT22*
1984	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
1985	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
1986	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X
1988	X	X	X	X				X	X	X	X	X	X	X	X		X	X		X	X	X
1989	X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
1990	X	X	X	X	-	X	-	-	X	X	X	X	X	X	-	-	X	X	O	-	-	-
1991	X	X	X	X	-	X	-	-	X	X	-	X		X	-	-		X		-	-	-
1992	X	X	X	X	-	X	-	-	X	X	-	-	X	X	-	-	X	X		-	-	-
1993		X	X	X	-	X	-	-	X	X	-	-			-	-	X	X		-	-	-
1994	X				-		-	-	X	X	-	-		X	-	-		X		-	-	-
1995	X	X	X	X	-	X	-	-	X	X	-	-	X	X	-	-	X	X		-	-	-
1996	X	X	X	X	-	X	-	-	X	X	-	-		X	-	-		X		-	-	-
1997	X	X	X	X	-		-	-			-	-	X		-	-	X			-	-	-
1998	X				-	X	-	-	X	X	-	-	X	X	-	-	X	X		-	-	-
1999		X	X	X	-	X	-	-	X	X	-	-			-	-		X		-	-	-
2000	O				-		-	-			-	-	X	X	-	-	X			-	-	-
2001		X	X	X	-	X	-	-	X		-	-	X		-	-	X	X		-	-	-
2002		X	X	X	-		-	-	-		-	-	O?	X	-	-	-			-	-	-
2003		X	X	X	-	X	-	-	X	X	-	-	-	O?	-	-	-	X		-	-	-
2004		X	X	X	-	X	-	-	X	X	-	-	-	-	-	-	-	X		-	-	-
2005		X	X	X	-	X	-	-	X	X	-	-	-	-	-	-	-	X		-	-	-
2006		X	X	X	-	X	-	-	X	X	-	-	-	-	-	-	-	X		-	-	-
2007		X	X	X	-	X	-	-	X	X	-	-	-	-	-	-	-	X		-	-	-
2008		X	X	X	-	X	-	-	X	X	-	-	-	-	-	-	-	X		-	-	-
2009		X	X	X	-	X	-	-	X	X	-	-	-	-	-	-	-	X		-	-	-
2010		X	X	X	-	X	-	-	X	X	-	-	-	-	-	-	-	X		-	-	-
2011		X	X	X	-	X	-	-	X	X	-	-	-	-	-	-	-	X		-	-	-
2012		X	X	X	-	X	-	-	X	X	-	-	-	-	-	-	-	X		-	-	-
2013		X	X	X	-	X	-	-	X	X	-	-	-	-	-	-	-	X		-	-	-
2014		X	X	X	-	X	-	-	X	X	-	-	-	-	-	-	-	X		-	-	-
2015		X	X	X	-	X	-	-	X	X	-	-	-	-	-	-	-	X		-	-	-
2016		X	X	X	-	X	-	-	X	X	-	-	-	-	-	-	-	X		-	-	-

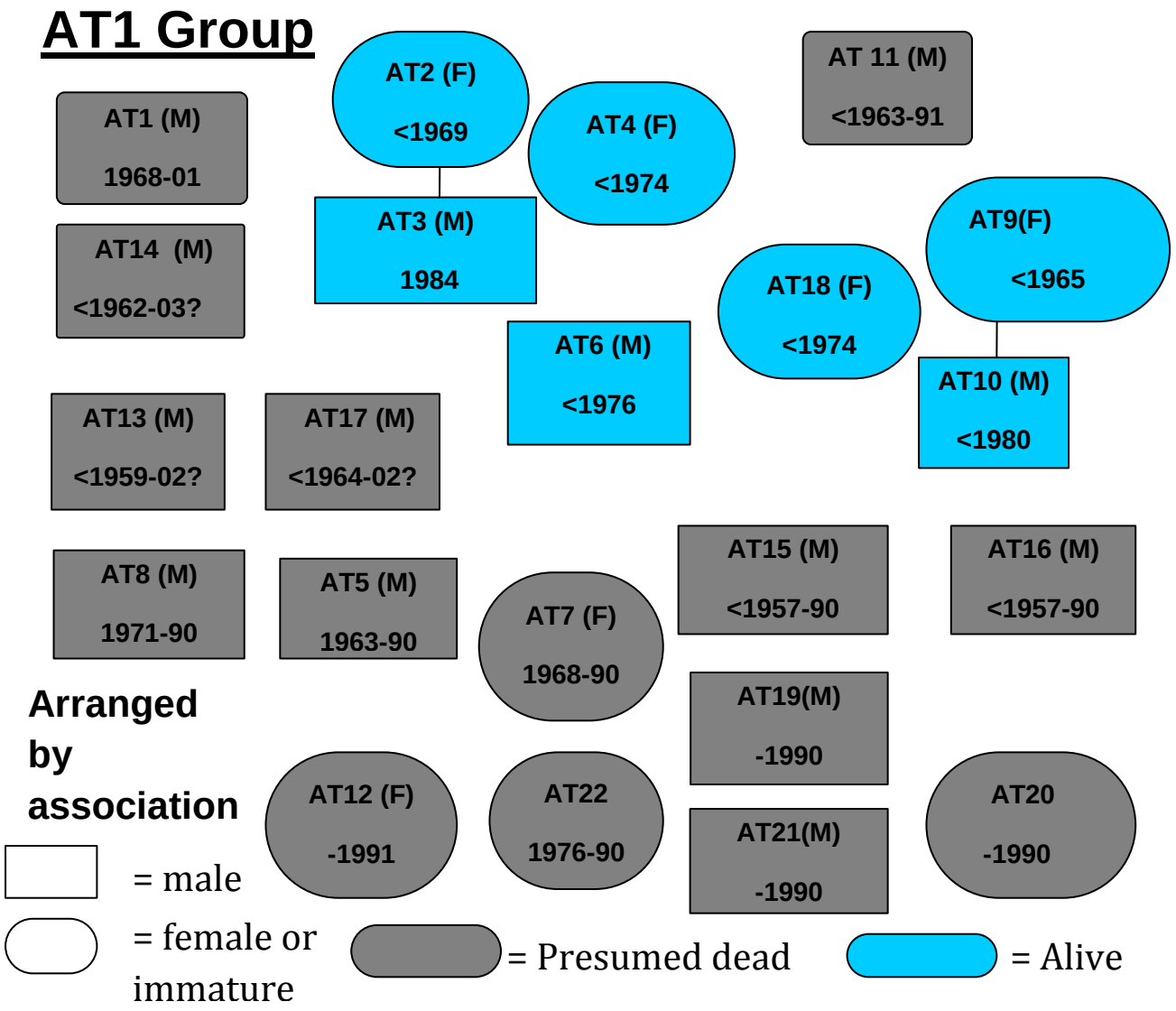

Figure 4. Diagrammatic representation of the AT1 (Chugach) transient population as it appeared in 1988 prior to the *Exxon Valdez* oil spill. Individuals are grouped by their associations, animals in dark grey are missing and presumed dead. Males are in square boxes. Years represent estimated or known birth and/or death years of individual whales.

Table 6. Summary of sightings of offshore killer whales from 2013 to 2016, with description of prey, if collected.

Year	Location	Number of whales photographed	Prey Samples
2013	Kachemak Bay, AK	16	Pacific sleeper shark
2015	Little Green Island, Prince William Sound, AK	25	Pacific sleeper shark
2015	Granite Cape, Kenai Fjords, AK	11	-
2015	Sea Otter Island, Kodiak, AK	17	-

Feeding Habits

Prey sampling and observation of kills by resident killer whales

A total of 222 scale samples were collected from 1991 to 2016 between April 1 and October 1 of each year, 39 were collected in the current study (2013-16). Of these samples, 106 were collected from Prince William Sound (Fig. 5) and 116 from Kenai Fjords (Fig. 6). Of the total collected, 6 were from a sockeye salmon (*Onchorhynchus nerka*), 88 were from Coho salmon, 32 were from chum salmon and 94 were from Chinook salmon. Despite their frequent abundance in the areas of prey collection, no samples were obtained from pink salmon (*O. gorbuscha*).

Although there is variability in the ease with which salmon lose their scales, the use of prey sampling has been demonstrated to be an effective method of examining feeding ecology of resident killer whales. The detailed fecal studies on resident killer whales in Puget Sound and British Columbia found Chinook and coho salmon to be primary prey despite the presence of other species (Ford et al. 2016). This work supported the results of many years of fish scale sampling at predation sites by Ford et al. (2009). Futhermore, there is evidence for preferential selection of the largest, oiliest (highest lipid containing) salmon (Chinook, followed by coho and chum) by resident killer whales in British Columbia (Ford and Ellis 2006). Resident killer whales frequently prey on salmon at depth but they often bring the fish to the surface as part of prey sharing behavior as determined by studies using d-tag attachment and closely tracking predation and feeding events (Wright et al. 2017). All of this work in British Columbia and Washington supports our interpretation of results from killer whale predation sites. However, we have initiated a fecal collection/analysis study component as a control for the prey sampling work for FY17-21.

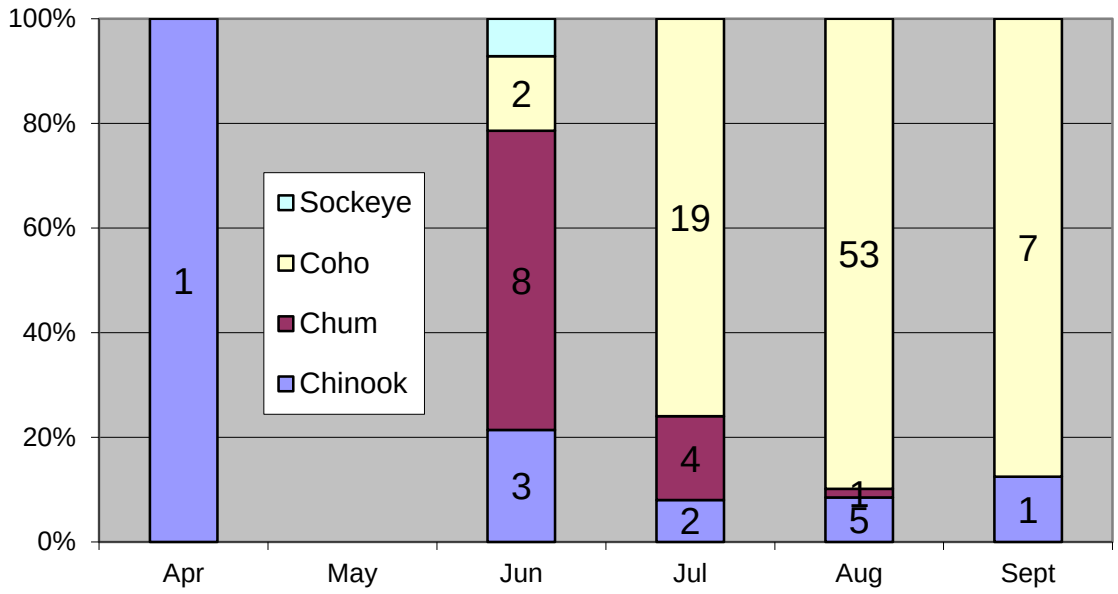

Figure 5. Species distribution of scale samples collected during southern Alaska resident killer whale predation events in Prince William Sound, Alaska, 1991-2016. Numbers represent sample sizes.

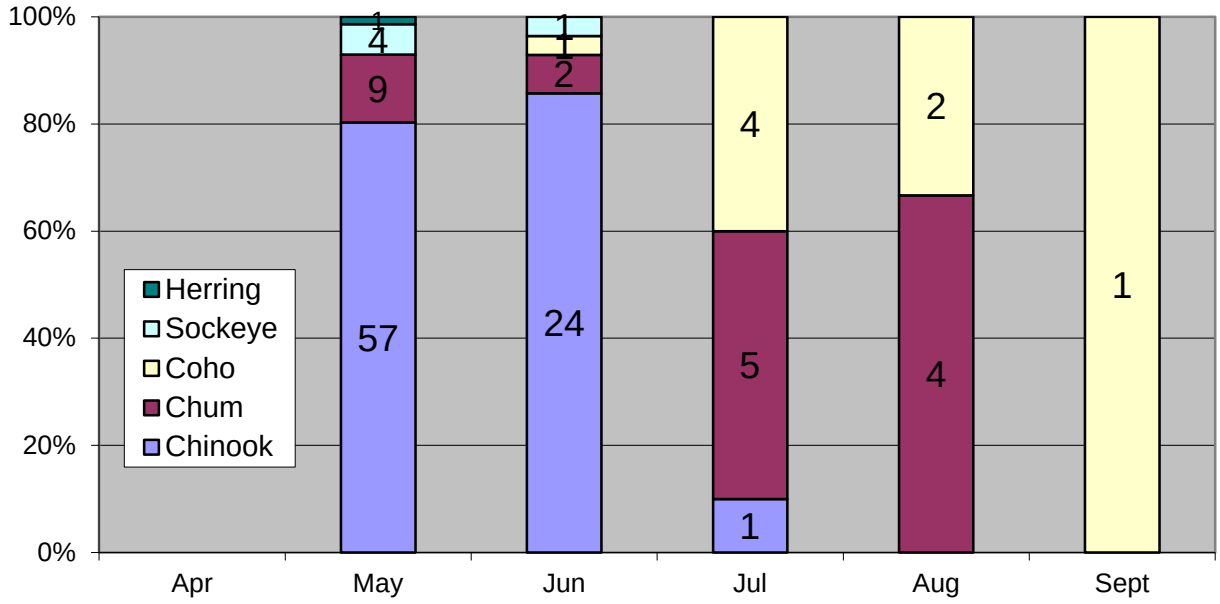

Figure 6. Species distribution of scale samples collected during southern Alaska resident killer whale predation events in Kenai Fjords, Alaska, 1995-2016. Numbers represent sample sizes.

Most of the Chinook predation events occurred in May or early June in Kenai Fjords. Unfortunately, there is limited data from this same spring period from Prince William Sound. The single late April sample from the Sound was a Chinook (Fig. 5) and of the 14 samples collected in June, three were Chinook while eight were chum. Although the sample size is small, in Kenai Fjords in July, August, and September our observations suggest that chum and Coho salmon are important prey. There has been an increase in chum salmon in the samples from recent years, which suggests an increase in the importance of this species in resident killer whale diet and is supported by the results of chemical analysis of skin and blubber (see chemical analysis below). Chum salmon become more available in summer in this region. In Prince William Sound in July, August, and September, there is some predation on Chinook and chum; however, predation appears to be primarily on coho after June. Coho become much more available in mid-summer to fall. Five of the six sockeye predation events occurred in Kenai Fjords in May and June but sockeye appear to be rarely taken overall. Often predation occurs within schools of pink salmon, but it appears whales are selecting other species swimming with the pink salmon. Over all the years of the study, there is no evidence of predation on pink salmon. Although fishing harvests in the region are primarily pink salmon, other species including coho and Chinook and chum salmon do make up part of the catch (Haught et al. 2017, Appendix D2).

Chemical analysis of resident killer whale blubber

We examined skin and blubber chemistry using samples randomly taken from the 10 most regularly encountered pods of whales in the study area over 12 years with little repeat in sampling of individuals. Stable isotopes values examined in skin change on a relative short term (over months). Because SI data were compared inter-annually, the few repeat samples of individuals over the years would have little effect on overall outcome. Over the past 12 years southern Alaska resident killer whales have revealed a long-term trend of sharply declining $\delta^{13}C$ and $\delta^{15}N$ values in the whales of our study area (Fig. 7). Isotopic changes in bowhead whale baleen were used by Schell (2000) who found evidence for declining productivity in the Bering and Chukchi seas.

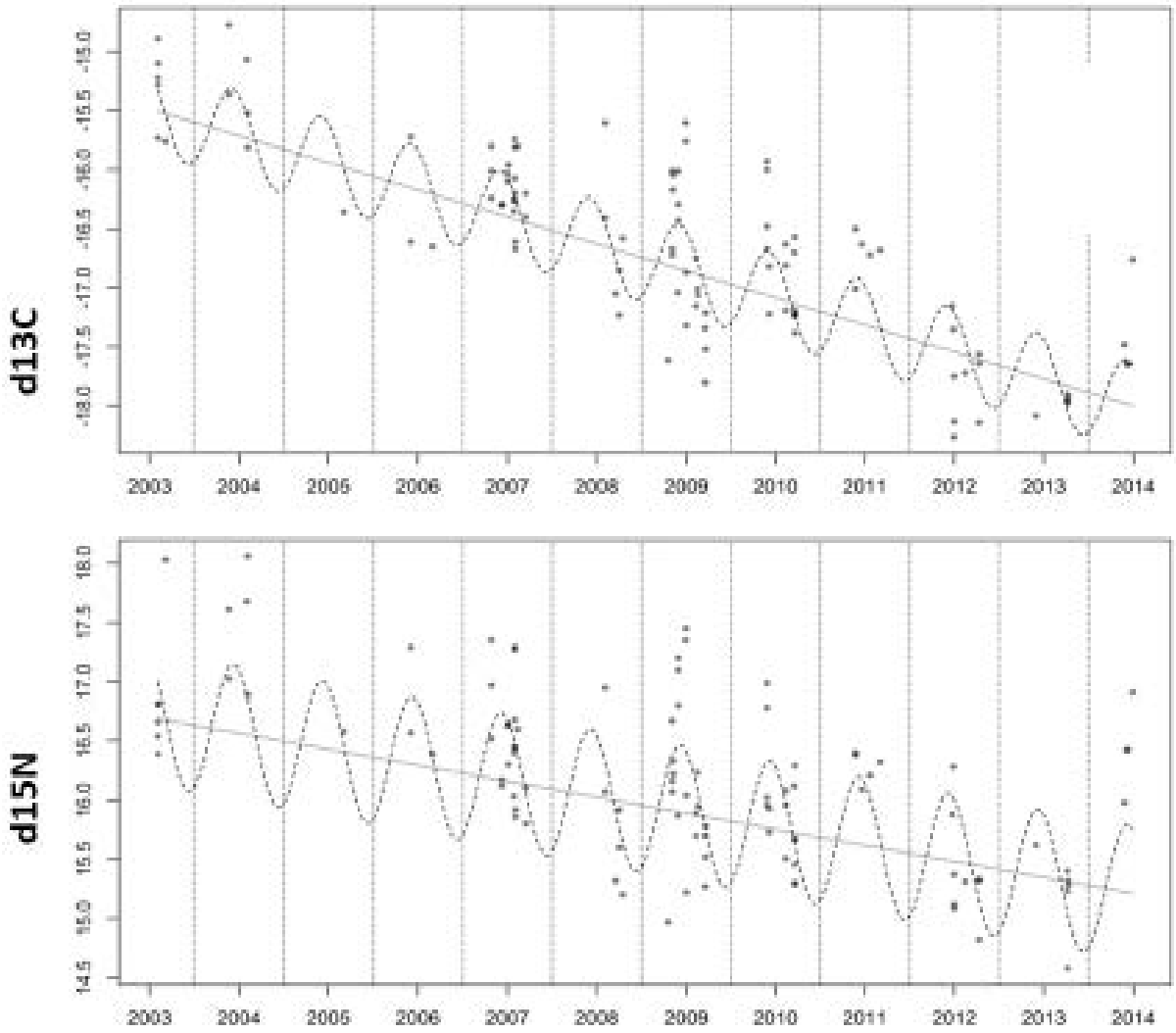

Figure 7. Changes in the $\delta^{13}C$ and $\delta^{15}N$ values from skin biopsy samples of southern Alaska resident killer whale (2003-2014). Dotted lines represent seasonal pattern of change within years. Each data point represents a single sample.

This change in $\delta^{13}C$ and $\delta^{15}N$ values of resident killer whales would likely reflect changes in prey composition or a dramatic shift in stable isotope values in the ecosystem. Carbon and nitrogen stable isotope values for Chinook salmon and coho salmon are substantially higher than values for pink salmon or chum salmon in southern Alaska. From samples taken across the seasons, the decline in isotopic values in the salmon eating whales suggests an increase in consumption of lower trophic feeding coho and chum salmon (Fig. 8)

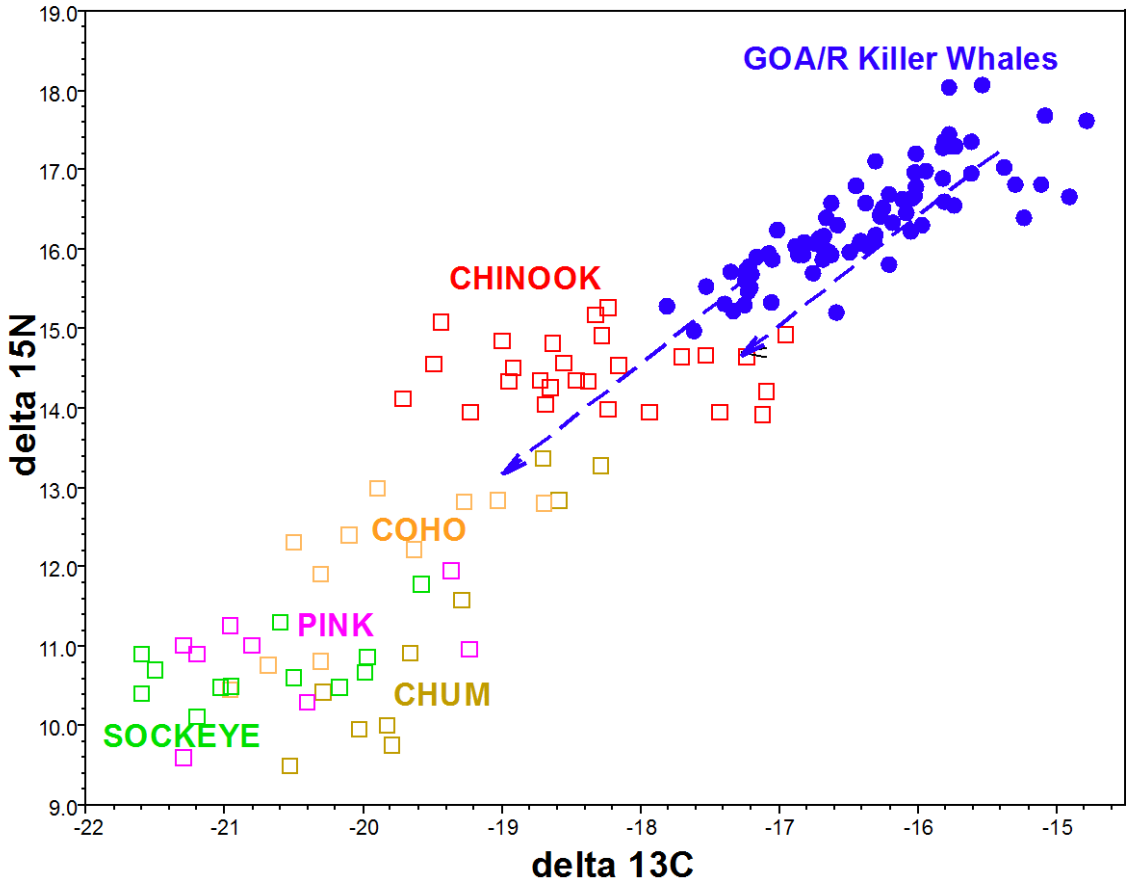

Figure 8. Isotopic values for the five salmon species found in the northern Gulf of Alaska in relation to values from skin of Gulf of Alaska resident (GOA/R) killer whales. Samples collected 1997-2013.

There is little evidence for a temporal change in isotope values in salmon sampled from 1997-2008 (Fig. 9). These relatively stable $\delta^{15}N$ values of the years for all species of salmon suggests that there has not been a significant ecosystem shift in the salmon food web as measured by stable isotope values, however, these data has not been adjusted for the Suess effect

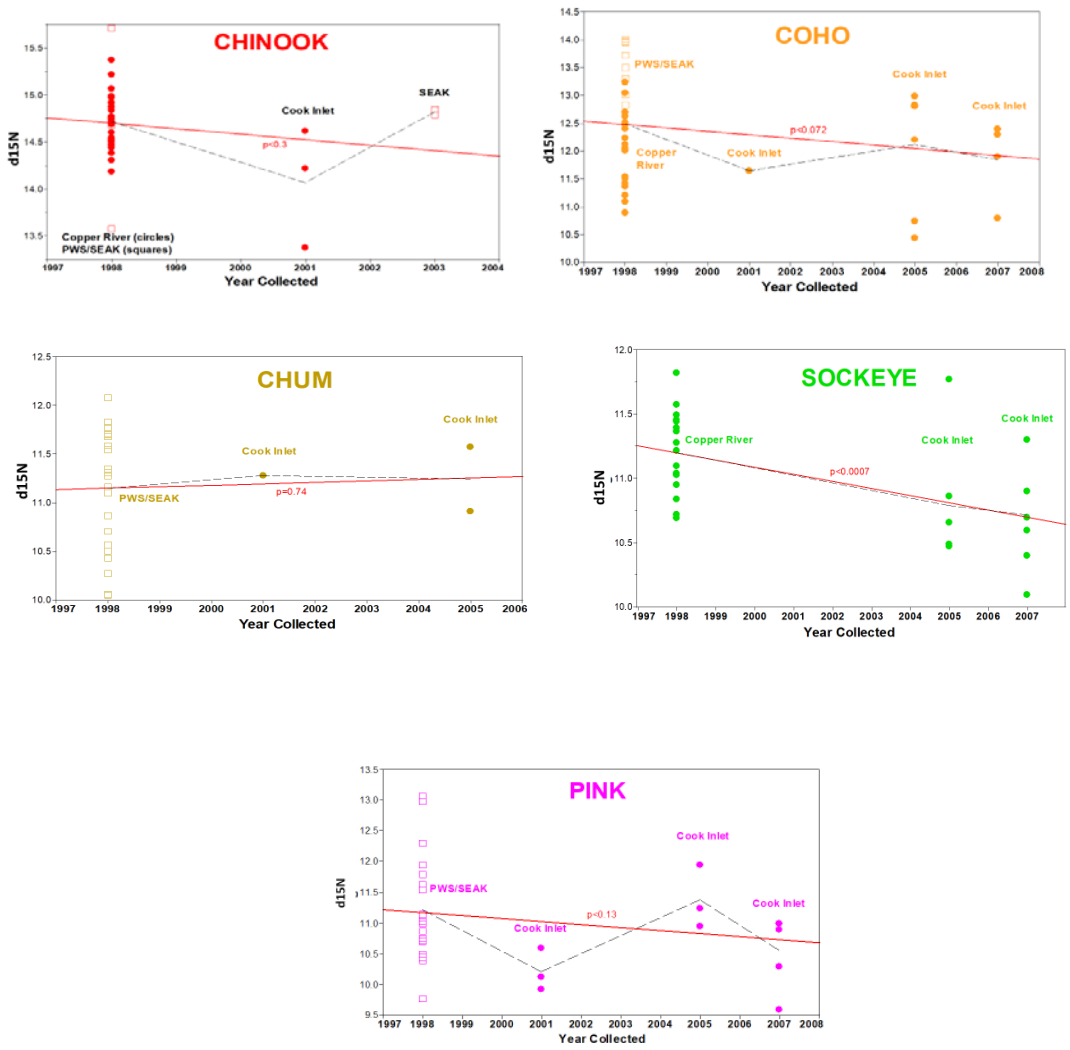

Figure 9. Levels of $\delta^{15}N$ in five species of Pacific salmon from samples taken 1997-2008. Each dot represents a single sample. Data courtesy of the Northwest Fisheries Science Center, National Marine Fisheries Service, Seattle, Washington.

The annual changes in $\delta^{13}C$ and $\delta^{15}N$ values have not been as dramatic for the endangered southern resident killer whale population of Puget Sound (Fig. 10). This suggests that significant changes in prey taken by killer whales and/or trophic changes in the food web have not occurred in this region and is supported by both scale sampling from predation sites (Ford et al. 2009) and from sampling of feces (Ford et al. 2016).

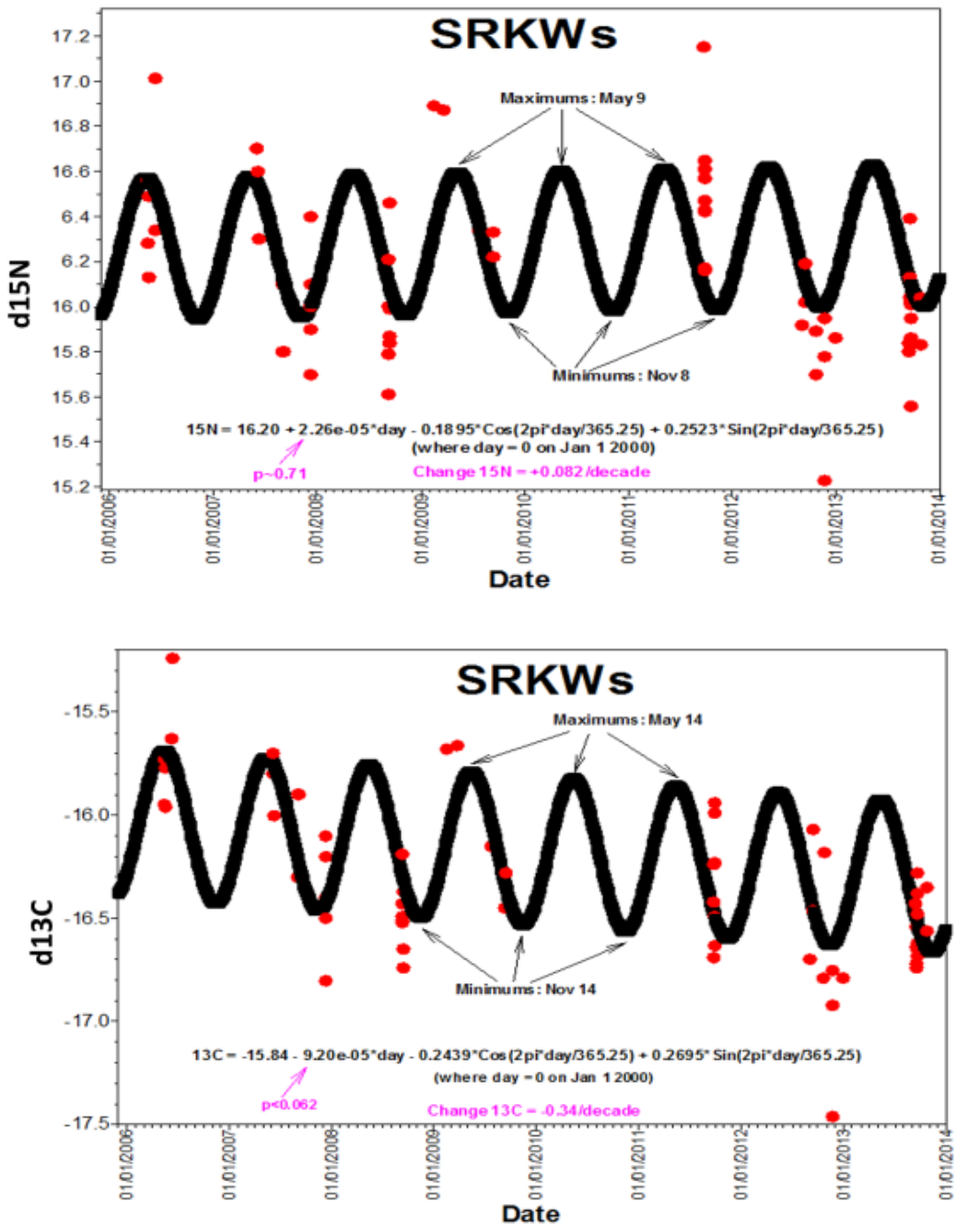

Figure 10. Changes in $\delta^{13}C$ and $\delta^{15}N$ values from skin biopsy samples of southern resident killer whale (2006-2014). Solid lines represent seasonal pattern of change within years. Indications are that the prey composition has remained relatively stable in this region where Chinook salmon are known to be important prey. Data provided by Gina Ylitalo and Brad Hanson, Northwest Fisheries Science Center, National Marine Fisheries Service, Seattle, Washington.

A sharp decline in contaminant levels (sum PCBs shown here) for southern Alaska resident killer whales (Fig. 11) also supports a probable change in diet for these whales over the past decade. Here we examined levels only in males where changes were not influenced by parturition and nursing. The typical reduction in PCB concentrations due to natural attrition is approximately 2% annually (Hickie et al. 2007), yet in southern Alaska residents the decrease in sum PCB levels ranged between 8 to 10% annually.

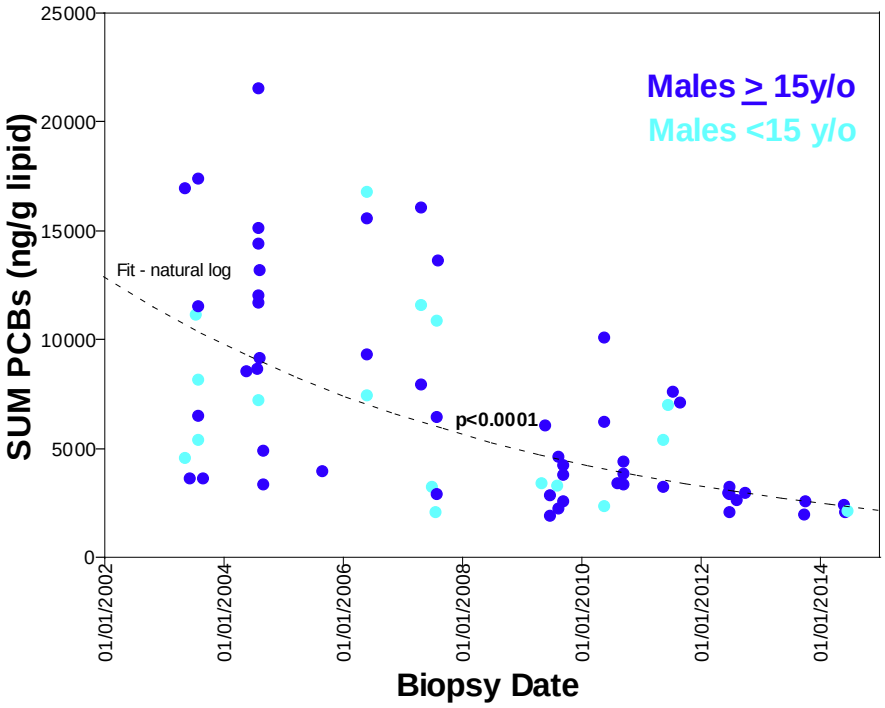

Figure 11. Decline in sum PCBs determined in blubber biopsy samples of male southern Alaska resident killer whales (2002-2015). Adult males (≥ 15 years of age) indicated as dark blue circles and juvenile males (< 15 years of age) as light blue circles. Each dot represents a single sample.

The PCB levels for the Puget Sound southern resident population declined closer to the 2%, which is the expected natural attrition rate (Fig. 12). This combination of factors, stable isotope and contaminant stability, suggests a relatively consistent diet for killer whales in the Puget Sound region over this period. It appears to be dominated by higher trophic level feeding Chinook salmon (Ford et al. 2009, Ford et al. 2016)

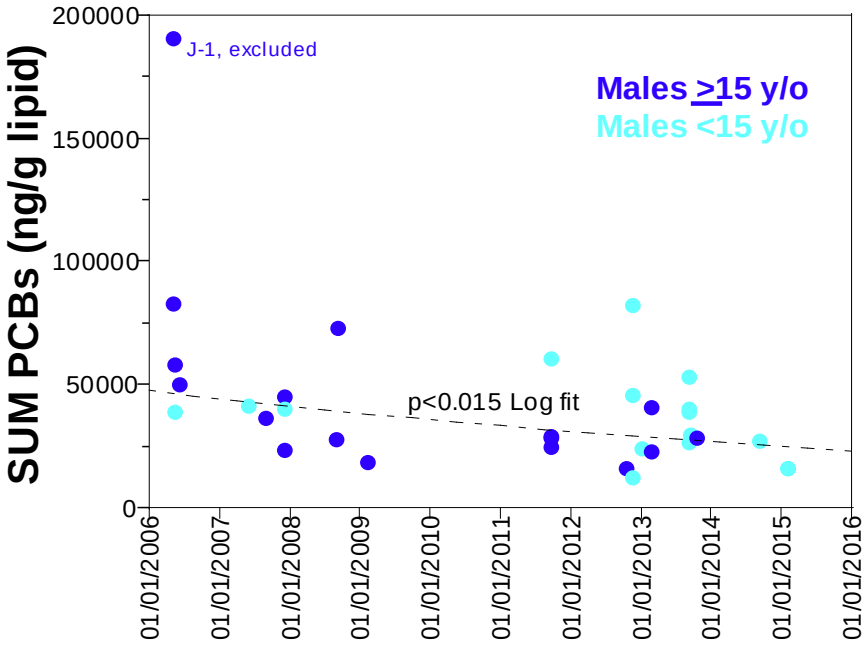

Figure 12. Changes in concentrations of sum PCBs determined in blubber biopsy samples of male Puget Sound southern resident killer whales (2006-2015). Adult males (≥ 15 years of age) indicated as dark blue circles and juvenile males (< 15 years of age) as light blue circles. Data provided by Gina Ylitalo and Brad Hanson Northwest Fisheries Science Center, National Marine Fisheries Service, Seattle, Washington.

Despite small some small sample sizes, sampling of fish scales from southern Alaska resident predation sites suggests an annual pattern of Chinook salmon predation in the spring followed by increasing predation on chum salmon and coho salmon as the summer and fall progress. This is supported by seasonal changes in the $\delta^{13}C$ and $\delta^{15}N$ in the blubber of the southern Alaska resident killer whales (Fig. 7) over the course of the season. There has also been a decline in the average annual stable isotope levels of the whales over the past 12 years. This change has not been observed in the southern residents of Puget Sound region over a similar time period. This would suggest either a change in $\delta^{13}C$ and $\delta^{15}N$ values across the entire Gulf of Alaska ecosystem or a change in feeding habits over the past 12 years. There is little evidence for a shift in salmon $\delta^{13}C$ and $\delta^{15}N$ values in recent decades that would be reflected in killer whale tissues, although recent data for fish samples have been lacking. Chum salmon, with lower isotope values have been increasing in the prey samples in recent years. Another indication that there has been a change in prey for southern Alaska killer whales in recent years is the unusually strong annual decline in PCB levels in southern Alaska residents (8%) while the southern residents of Puget Sound have only had sum PCB declines of about 2% per year. The 2% annual decline is more in line with the

expected rate of degradation over time. This change in prey composition may be due to the decline of abundance of Chinook salmon in the region over the past decade or due to the expanded nutritional needs of what is an increasing population of resident killer whales except for the oil spill-affected AB pod. Unfortunately, there are few good measures of prey abundance, e.g., numbers of feeding Chinook or numbers of returning coho salmon that can be tracked annually.

Marine mammal predation by transient killer whales

Feeding habits of transient killer whales has been a lesser priority from earlier phases of the long-term study when significant additional field time was available and allotted to this aspect of the work. We did observe three predation events and a single harassment/attempted predation during this four-year phase of the study (Table 7). The AT1 transient predation was on a harbor seal, their primary prey from previous years' observations and the three Gulf of Alaska transient events all involved Dall's porpoise.

Table 7. Marine mammal kills by killer whales observed during the study period, 2013-2016.

Date	Species	ID Method	Whale ID
2013/06/17	Harbor seal	genetic ID	AT 2,3,4
2013/09/01	Dall's porpoise	observation	AT73,80,81
2014/05/23	Dall's porpoise	observation harass	AT128+
2015/05/13	Dall's porpoise	observation	AT72,73,80,81,juv

In overall years of the study we have observed 83 predation and attempted predation events by Gulf of Alaska transients and 93 such events with AT1 transients (Table 8). This time the majority of predation for GOA transients have been on Steller sea lions, although in recent years we have observed more kills of Dall' s porpoise, the second most common prey item. The AT1 transients are seldom seen due to their low numbers (7 remaining) and apparent tendency to spend a majority of their time near tidewater glaciers where there are an abundance of harbor seals and where we seldom work. Working in glacial areas that are widespread is difficult and would preclude monitoring the oil spill damaged and recovering AB pod and other southern Alaska residents on which our population dynamics work depends. We suspect the AT1 transients remain primarily harbor seal predators with some offshore foraging for Dall's porpoise.

Most of the predation by the Gulf of Alaska transients on Steller sea lions occurred near rookeries and haulouts and adjacent feeding areas where concentrations of sea lions occurred. Most harbor seal kills by AT1 transients occurred beneath the water's surface and were detected by the appearance of blubber fragments, hair, and oil on the surface. Seabirds often investigated the kill sites and sometimes alerted us to their

occurrence. When visual identification of prey was uncertain, we relied on genetic analysis of tissue. In contrast, Dall's porpoise kills (n=13) involved highly visible surface chases. For the AT1 transients, all but three harbor seal kills occurred during near-shore foraging or near glaciers, and all Dall's porpoise kills occurred during offshore foraging. Although AT1 killer whales have harassed Steller sea lions on 14 occasions, we have never seen a focused attack or predation and there is no strong evidence that they consume them.

Table 8. Summary of predation and harassment events for Gulf of Alaska and AT1 transient killer whales.

Gulf of Alaska (GOA) Transients 1984-2015			
	Kills	Harass	Total
Steller sea lions	13	29	42
Dall's porpoise	7	5	12
Sea otter	1	5	6
Harbor seal	2	0	2
Birds	9	6	15
Humpback	0	2	2
Harbor porpoise	2	0	2
UnID	2	0	2
TOTAL	**36**	**47**	**83**

Note: Three of the Steller sea lion kills were observed in Kodiak Island waters. One harbor seal kill and two harbor porpoise kills were observed in Kachemak Bay.

AT1 transients 1984-2015			
	Kill	Harass	Total
Harbor seal	17	13	30
Dalls Porpoise	13	8	21
Steller sea lion	0	14	14
Harbor porpoise	2	0	2
Northern fur seal	1	0	1
UnID marine mammal	9	3	12
Humpback whale	0	8	8
Sea otter	0	3	3
River otter	0	1	1
Salmon	0	1	1
TOTAL	**42**	**51**	**93**

In Gulf of Alaska waters all prey samples from remains of kills by offshore killer whales have consisted of the livers of Pacific sleeper sharks (Ford et al. 2011). In this study the two samples of offshore prey retrieved were all Pacific sleeper shark (Table 6). It appears groups of offshore killer whales make forays into these northern Alaskan waters specifically to prey on these sharks.

CONCLUSIONS

The resident AB pod continues a very slow recovery that is still not complete nearly three decades after the oil spill. The oil spill-impacted AT1 population of transient killer whales has been stable at 7 individuals for nearly a decade, but has produced no calves and is apparently headed for extinction. However, the broader population of resident killer whales continues to increase at a rate of about 3% as has been the case since this long-term study began 32 years ago. Because pods of these whales have grown and split, and ranges of some pods have changed our examination of population dynamics in the future will likely focus on changes in matrilineal groups (building blocks of pods) rather than entire pods. We estimate there is now a minimum of 1062 whales in the southern Alaska resident killer whale population. Encounters with the offshore ecotype of killer whale remain infrequent with only 4 encounters recorded during the four-year study period.

The concurrent decline of stable isotope and contaminant values (PCBs) in the southern Alaska resident killer whales while salmon $\delta^{15}N$ remain relatively constant, suggests a decrease in Chinook salmon (high trophic level predator/higher $\delta^{13}C$ and $\delta^{15}N$ values) in diet and increase in Coho and/or chum salmon (lower trophic level predators/lower $\delta^{13}C$ and $\delta^{15}N$ values). This trophic shift in southern Alaska resident killer whales has also been observed over the course of the season when prey sampling suggests more Chinook are taken in spring and that more chum and then Coho in summer and fall. The annual pattern may represent a reduction in Chinook availability during the twelve year period of our measurements, but may also be evidence of the increasing resident killer whale population requiring a larger prey base and increasingly preying on the less desirable, smaller and lower oil content salmon species. Analysis of FA data is ongoing and will contribute to our knowledge of killer whale foraging ecology.

Although we have not focused on marine mammal predation by transient killer whales in recent years, it appears the remaining AT1 killer whales primarily prey on harbor seal predators with Dall's porpoise also an important component of the diet. Gulf of Alaska transients appear to focus on Steller sea lions and recently we have observed an increasing number of predation events on Dall's porpoise. It appears offshore ecotype killer whales make occasional forays into the study area to feed on Pacific Sleeper sharks.

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Alaska the success we enjoyed in the field would not have been possible. We look forward to continuing our cooperative effort in the future. To the numerous individuals that have aided in field data collection and data input over the years, we offer our sincere thanks. The views expressed here are our own and do not necessarily represent those of the *Exxon Valdez* Oil Spill Trustee Council.

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Seasonal and pod-specific differences in core use areas by resident killer whales in the Northern Gulf of Alaska

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ABSTRACT

The resident killer whale is a genetically and behaviorally distinct ecotype of killer whale (*Orcinus orca*) found in the North Pacific that feeds primarily on Pacific salmon (*Oncorhynchus spp .*). Details regarding core use areas have been inferred by boat surveys, but are subject to effort bias and weather limitations. To investigate core use areas, 37 satellite tags were deployed from 2006 to 2014 on resident killer whales representing 12 pods in the Northern Gulf of Alaska, and transmissions were received during the months of June to January. Core use areas were identified through utilization distributions using a biased Brownian Bridge movement model. Distinct differences in these core use areas were revealed, and were highly specific to season and pod. In June, July, and August, the waters of Hinchinbrook Entrance and west of Kayak Island were primary areas used, mainly by 3 separate pods. These same pods shifted their focus to Montague Strait in August, September, and October. Port Gravina was a focal area for 2 other pods in June, July, and August, but this was not the case in later months. These pods were responsible for seven of eight documented trips into the deeper fjords of Prince William Sound, yet these fjords were not a focus for most groups of killer whales. The seasonal differences in core use may be a response to the seasonal returns of salmon, though details on specific migration routes and timing for the salmon are limited. We found strong seasonal and pod-specific shifts in patterns between core use areas. Future research should investigate pod differences in diet composition and relationships between core area use and bathymetry.

1. Introduction

Resident killer whales are a genetically distinct piscivorous ecotype of killer whale found only in the North Pacific Ocean (Hoelzel et al., 1998; Morin et al., 2010; Parsons et al., 2013). They have diverged behaviorally, genetically, and acoustically from other sympatric ecotypes of killer whales, including the 'transient' killer whale ecotype which eats mammals (Ford et al., 1998; Heimlich-Boran, 1988), and the 'offshore' killer whale ecotype which preys on sharks and other fishes (Ford et al., 2011). The 'resident' ecotype has been observed feeding exclusively on fish, primarily Pacific salmon (*Oncorhynchus sp.*), and has never been observed feeding on mammals or sharks (Ford et al., 1998, 2016; Saulitis et al., 2000). Scale and tissue samples collected during predation events imply Chinook (*Oncorhynchus tshawytscha*), coho (*Oncorhynchus kisutch*), and chum salmon (*Oncorhynchus keta*) as primary prey for resident killer whales in the Northern Gulf of Alaska (Matkin et al., 2013; Saulitis et al., 2000).

Resident killer whales typically spend their entire lives within their natal matrilines, which consist of a female, all of her adult offspring, and any of the offspring of females born to her (Bigg et al., 1990; Matkin et al., 1999). Dispersal from the natal matriline is rare in Washington, British Columbia and Alaska (Barrett-Lennard, 2000; Parsons et al., 2009). Killer whale pods are defined as social units consisting of related matrilines that are together during more than 50% of sightings, and are believed to have common lineage (Bigg et al., 1990). The relatedness of calls within these pods and matrilines parallel genetic relatedness (Yurk et al., 2002).

Pod structure for resident killer whales has been very well documented in three different populations, including the southern residents in Puget Sound and the northern residents in British Columbia. The third, and the subject of this study, is a population known as the southern Alaska residents. This population spans from southeastern Alaska to Kodiak, and includes approximately 700 whales (Matkin et al., 2014).

Killer whale pods and matrilines transmit cultural traditions through generations, including acoustic repertoires and call types (Filatova et al., 2015; Ford, 1991; Yurk et al., 2002). Cultural transmission is also believed to contribute to similarities in space use

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between groups (Similä et al., 1996), hunting and feeding techniques (Guinet and Bouvier, 1995; Similä and Ugarte, 1993), and beach rubbing (Rendell and Whitehead, 2001). Pod-specific core use areas have been documented in the southern resident killer whale population (Hauser et al., 2007), but have yet to be reported in Alaskan waters. Core use areas are important to monitor, as they illustrate temporal trends and inform potential variation within a population.

Satellite telemetry is a useful tool in describing core use areas, and is a method that is less subject to bias than boat surveys, which are limited by weather, survey locations, and daylight hours. In the Puget Sound, satellite telemetry is currently being used to assess important winter habitat use of the declining southern resident killer whale population (Northwest Fisheries Science Center, NOAA, 2014). Satellite telemetry has given insight into winter areas for that stock, and has enabled researchers to re-sight tagged animals and gather winter predation data that were previously lacking (Northwest Fisheries Science Center & NOAA, 2014). Telemetry has also proven to be a useful tool in describing important areas for many other cetacean species, such as false killer whales (*Pseudorca crassidens*), narwhals (*Monodon monoceros*), humpback whales (*Megaptera novaeangeliae*), and Hectors dolphins (*Cephalorhynchus hectori*) (Baird et al., 2013; Heide-Jørgensen et al., 2002; Kennedy et al., 2014; Rayment et al., 2009).

As an apex predator, resident killer whales are important to monitor for both conservation and management, particularly due to their strong preference for salmon. In the present study, we use the location data from 37 deployed satellite tags on killer whales in the northern Gulf of Alaska to assess core use areas. We document seasonal use differences from June through October for certain pods, and describe variation in use between pods. We hypothesize that distinct differences occur in core areas throughout the seasons, in response to prey availability and that pod-specific use of the region is non-random.

2. Materials and methods

2.1. Study area and animal selection

The study area spanned the northern Gulf of Alaska from Southeast Alaska to the Alaska Peninsula (Fig. 1). The bays and passes of Prince William Sound, the Kenai Coast, Kodiak Island, and Cook Inlet are glacially carved and therefore relatively deep (300–500 m), and experience strong tidal currents (Halverson et al., 2013). The coastwise portion of this study area includes the continental shelf, which extends from 30 to 170 km offshore. The shelf ranges in depth from 100 to 300 m in this region, and is subject to a general westward flow of the Alaska Coastal Current (Royer, 1981). Strong downwelling conditions in winter promote inflow into Prince William Sound through Hinchinbrook Entrance and outflow through Montague Strait, but this pattern is less distinct in the summer months as offshore downwelling conditions relax (Halverson et al., 2013).

Thirty-seven satellite tags were deployed on killer whales amongst 14 pods between 2006 and 2014 in Prince William Sound and Kenai Fjords (Table 1). Given the extremely rare dispersal from matrilines (Barrett-Lennard, 2000), the movement of one individual was taken to be representative of the movements of its entire matriline, and representative of its pod. Tagging locations were opportunistic, performed during photo identification surveys in Prince William Sound and Kenai Fjords (Fig. 1).

2.2. Tagging method

Whales were tagged with low impact minimally percutaneous external-electronics transmitter (LIMPET) satellite tags (Andrews et al., 2008). Tag designs were Wildlife Computers (Redmond, WA) SPOT 5 (AM-240, B, and C), and SPLASH10 (AM-266A and AM-292A). Tags were deployed by crossbow or air rifle at a distance of 6–20 m from a 12-m survey vessel. Two 6.5 cm long titanium darts equipped with backward-facing barbs were used to anchor the tags in the connective tissue of the dorsal fin (Andrews et al., 2008). These transmitters sent ultra-high frequency (UHF) radio signals to Argos receivers onboard weather satellites.

To conserve power, transmissions were limited to whale surface time by a submersion sensor, but otherwise transmitted during all hours of the day. If tags lasted more than 50 days they were programmed to transmit every other day afterward (3 tags fit this category). If tags lasted more than 65 days, they were programmed to transmit every 5 days (2 tags fit this category).

2.3. Data analysis

Locations were calculated by the Argos system using the method of least squares, and each location was assigned a location class. Location classes (LC) 3, 2, and 1 are assigned an accuracy estimate by Argos, with the 68th percentile error ranging from 0.25 to 1.5 km, while the remaining LCs (0, A, B, and Z) are not assigned an error. All location data were subsequently processed with the Douglas Argos Filter, based on location class and realistic movement parameters, including turning angles and distance ratios between positions (Douglas et al., 2012). For core use analyses, the first 24 h of data were removed from each deployment to minimize potential tagging site bias. Twenty four hours were considered sufficient because killer whales can make mean daily movements of over 100 km (Matthews et al., 2011; Williams and Noren, 2009).

Locations of core use areas were estimated using kernel density estimation and measured with utilization distributions (UDs). UDs are defined as the minimum area encompassing a certain probability of relocation (Kie et al., 2010; Seaman and Powell, 1996). Core use areas are defined as the 50% UD probability contour (Fieberg and Kochanny, 2013; Kie et al., 2010; Schuler et al., 2014). One challenge with telemetry data and kernel density estimators is the potential for results to be biased by temporal and spatial autocorrelation. To minimize autocorrelation, we estimated UDs using a biased Brownian Bridge model. This model improves the traditional kernel density algorithms by placing calculated relocation probability between locations that satisfy limited time parameters, not only at received locations (Horne et al., 2007). This lessens the dependence on each location and provides a more accurate representation of the used space.

We calculated UDs for each pod, for each month, and each year, using the R package adehabitatHR (Calenge, 2011). A user-defined grid of 1 million pixels was established over the entire area of received locations, in order to assign the UD densities. However, cell size is reported to have little effect on the density distribution (Calenge, 2011). To adjust for variation in sample size due to tag transmission duration, UDs were first calculated for each animal, and the subsequent density values were weighted by the number of days of tag deployment. After summing the individual densities, values were standardized so that the probability across the grid still summed to a value of 1.

To assess variability in use, we examined the core use (50% UD) by pod, month, and year. Probability polygons were created which were imported into QGIS for analysis and comparison of UD sizes for each month, each pod, and each year. Each pod was identified by a 2-letter code and in some cases with an additional number. To examine temporal variation in the areas used, we examined seasonal and interannual variability within pods that had large sample sizes (AJ pod, 10 deployments, 4354 locations, 348 days), or by pooling pods, e.g. AD16 and AK pods, which are known to be closely related (4 deployments, 1155 locations, 166 days). The land portions of the UD's were eliminated for core use areas size calculations.

To limit erroneous calculations of short-term movements, we used speed and distance calculations only for positions that were separated by more than one hour and less than six hours. Speed calculations from Argos positions that are less than one hour apart can be greatly exaggerated by erroneous positions, and positions that are more than six

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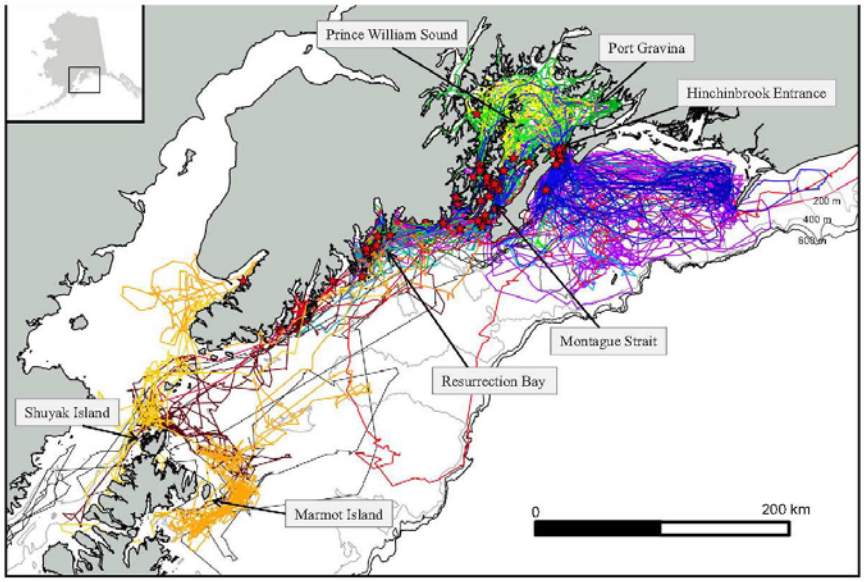

Fig. 1. Study area for resident killer whales (*Orcinus orca*) with tag deployment locations (red stars) and 200 m, 400 m, and 600 m bathymetry contours. Tracklines are colored by pod, AB (blue), AD5 (gold), AD16/AK (green), AE (yellow), AF/AG (black), AI (light blue), AJ (purple), AX48 and AY (dark red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Number of tagged animals, locations, and transmission days from tagged resident killer whales (*Orcinus orca*), by pod, 2006–2014.

Pod	Tag Deployments	Filtered Locations	Total days	Months represented
AB	5	1287	113	6,7,8,9,10,11
AD5	3	746	127	6,7,10,11,12
AD16/AK	4	1155	166	6,7,8,9,10
AE	2	357	73	8,9,10
AF/AG	3	884	55	7,8,9
AI	3	831	47	8,9,10,11
AJ	10	4534	348	6,7,8,9,10,11,12
AX48	4	890	100	6,7,8
AY	2	847	77	6,7
AW	1	250	35	8,9
Total	37	11,781	1141	6,7,8,9,10,11,12

hours apart are likely to miss non-linear movements.

3. Results

Transmissions were received from 37 deployments on killer whales for a total of 1141 transmission days between 2006 and 2014. Transmissions were received between the months of June and December in all years and from one tag in January 2011 (Table 1). The majority (91.9%) of transmissions were received between June and October. The mean number of days that each tag transmitted was 26.1 days. Of the locations that passed the Douglas Argos Filter, none were received from beyond the continental shelf break (Fig. 1). Median short-term movements in this study were estimated at 4.43 km/h, which extrapolates to 106 km/day.

Strong seasonal differences in core use areas were evident, particularly between summer and fall months. Hinchinbrook Entrance was a strong focal area for the AJ pod during June, July, and August, but was used much less in September and October (Fig. 2). Montague Strait was heavily used in August, September, and October, but not in earlier months (Figs. 2,3). The waters west of Kayak Island saw consistent use in June, July, and August, but less use in September and October (Fig. 2). Port Gravina was a focal area for two pods during June, July, and August, but had no evident use during September or October (Fig. 3).

Differences in core use were also evident between individual pods. The AB, AI, and AJ pods accounted for most of the use in Hinchinbrook Entrance and most of the use in Montague Strait, and they were the only pods that demonstrated regular use of waters west of Kayak Island (Fig. 4). The waters west of Kayak Island are likely important, as 12 out of the 16 tagged animals from the AB, AI, and AJ pods made at least one visit to this area. The AB, AI, and AJ pods were also the primary pods to use offshore areas ranging near the shelf break. The AD16 and AK pods did not use offshore waters, and were the primary users of the northern edges of Prince William Sound, including the long glacially carved fjords (Fig. 1). These two pods were responsible for seven out of the eight trips recorded into these long fjords. The AE pod used the inside waters of Prince William Sound, but were not observed using the long fjords, nor offshore waters (Figs. 1,4). The AD5 and AY pods were the primary pods to use Resurrection Bay and the waters adjacent to Shuyak and Marmot Islands, near the northern end of Kodiak Island (Figs. 1 and 4).

A high percentage of positions in Montague Strait were located within a glacially carved trench that is 200–300 m deep (Fig. 5). Within Montague Strait, 1346 of 2035 locations (66%) were in waters between 200 and 300 m deep, even though this only represents 21% of the possible area. The 200-m isobath creates a clear boundary for the majority of these locations. Furthermore, the AB, AI, and AJ pods were the primary pods to concentrate over these deeper waters (Fig. 5). This dynamic was not observed in other areas of Prince William Sound or the northern Gulf of Alaska.

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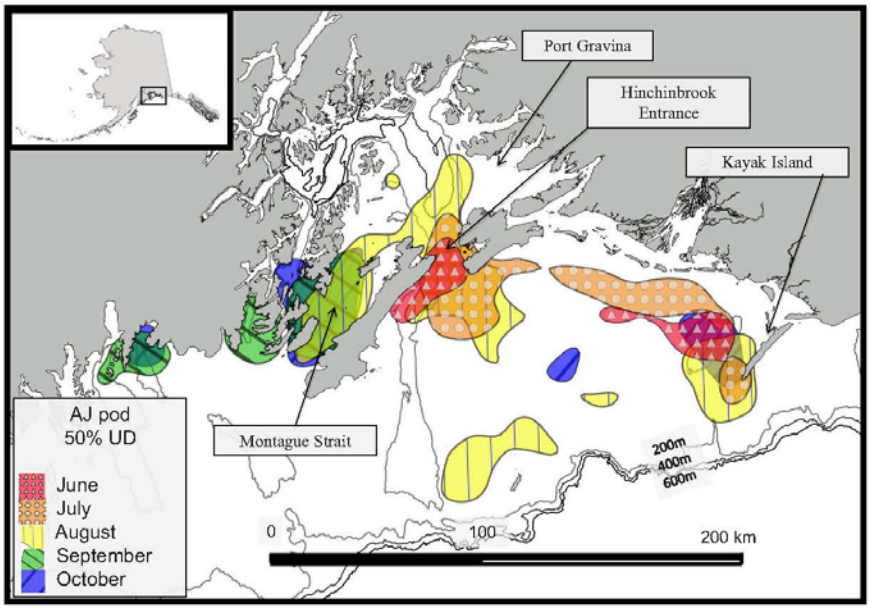

Fig. 2. Monthly variation in space use by AJ pod of resident killer whales (*Orcinus orca*). Core area use, or 50% UD, is displayed by month. Monthly 50% UD is displayed with color and symbols; June (red with triangles), July (orange with circles), August (yellow with vertical lines), September (green with back diagonal), October (blue with forward diagonal). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Differences in core use areas occurred seasonally for the tagged resident killer whales in the northern Gulf of Alaska, and these differences were at times specific to individual pods. We presume that the observed seasonal shifts in core use areas (Montague Strait, Hinchinbrook Entrance, Port Gravina) may be related to the specific timing of returns of Chinook, chum and coho salmon to their natal spawning streams, and the congregation of Chinook salmon while foraging in nearshore waters. Salmon perform highly predictable seasonal returns to their natal streams, and Chinook, coho, and chum salmon have been shown to comprise a major portion of the summer diet for resident killer whales in south central Alaska (Matkin et al., 2013; Saulitis et al., 2000). The arrival of resident killer whales and salmon has been shown to occur concurrently in British Columbia (Hanson et al., 2010), and Chinook and coho salmon have been shown to dominate the summer diet of resident killer whales in that area as well (Ford and Ellis, 2006; Ford et al., 2016). Survival rates for resident killer whales in British Columbia have been linked with abundance of Chinook salmon (Ford et al., 2010). In Alaska, prey samples have been collected at two of the high-use areas (Hinchinbrook Entrance and Montague Strait) noted in this study, and were dominated by scales from Chinook, coho, and chum salmon (Matkin et al., 2013; Saulitis et al., 2000). Seasonal dietary shifts from Chinook to coho have been documented in both Alaska and the Pacific Northwest (Ford et al., 2016; Matkin et al., 2013).

The Alaska Department of Fish and Game reports peak chum return timing to occur in late June in Prince William Sound (ADFG, 2002), which could be partially responsible for the high use of Hinchinbrook Entrance in early summer. Chum salmon scales from predation by resident killer whales have been collected in Hinchinbrook entrance in June (Matkin et al., 2013). Hinchinbrook Entrance is one of the two main entrances to Prince William Sound, and is the main influx of water into the sound (Halverson et al., 2013). Scale collection during predation events has not occurred in Port Gravina, but the timing is consistent with Chum salmon runs in the area. Further collection of scat or scales and flesh from predation events is warranted.

The high use in Montague Strait in late summer and fall coincides with large congregations of adult Pacific herring (*Clupea pallasi*) and the Humpback whales (*Megaptera novaeangliae*) that prey on them (Moran et al., 2015). Although herring are important in the diet of killer whales in Norway and Iceland, the technique for hunting them is evident from the surface (Samarra and Foote, 2015; Similä et al., 1996). Herring predation is very rare for well studied killer whales in the North Pacific based on observations from surface kill remains and scat analysis (Ford and Ellis, 2006; Ford et al., 2016; Saulitis et al., 2000). It is possible that this aggregation of herring attracts feeding Chinook and coho salmon.

Pod specific core use preferences described in the present study may be the result of cultural transmission of learning through generations, as individuals swim with their mother or close relatives throughout their lives (Bigg et al., 1990). Cultural transmission has been documented amongst killer whale acoustic repertoires, foraging strategies, and habitat preferences (Guinet and Bouvier, 1995; Hauser et al., 2007; Similä and Ugarte, 1993; Yurk et al., 2002). Similar pod-specific core use patterns were noted in the San Juan Islands for southern resident killer whales (Hauser et al., 2007). Another possible cause of these patterns could be competition, but this has not been observed. To the contrary, killer whale pods are often attracted to one another for social and reproductive reasons. They have been shown to mate outside of their natal pod, particularly with pods that are least genetically similar (Barrett-Lennard, 2000). Furthermore, closely related pods in this study demonstrated similar patterns of space use. AB, AI, and AJ pods share the 'northern resident' haplotype (Parsons et al., 2013), and are the only pods shown to use offshore waters west of Kayak Island and

Appendix 1
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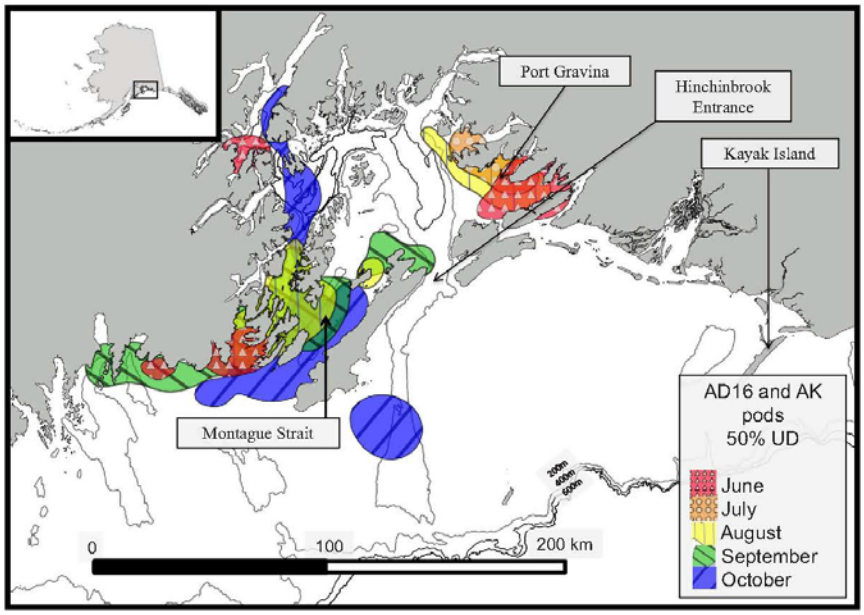

Fig. 3. Monthly variation in space use by combined AD16 and AK pods of resident killer whales (*Orcinus orca*). Core area use, or 50% UD, is displayed by month. Monthly 50% UD is displayed with color and symbols; June (red with triangles), July (orange with circles), August (yellow with vertical lines), September (green with back diagonal), October (blue with forward diagonal). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Hinchinbrook Entrance. AD16 and AK pods share the 'southern resident' haplotype, and are the only pods to use upper fjords and to focus on nearshore areas. The very large linear range difference between the unrelated AE pod (roughly 200 linear km) which has the 'southern resident' haplotype, and the AF and AG pods (1300 linear km) which have the 'northern resident' haplotype, is striking. While this difference could be attributed to diet differences, we suggest that these differences in linear range stem from social and reproductive behavior (Matkin et al., 1997).

Bathymetry appears to be important in some core use areas, and should be explored further. Our results show that the deeper waters (200–300 m) of Montague Strait and Port Bainbridge are important during summer and fall, particularly for the AB, AI, and AJ pods. Bathymetric features have been found to be preferential habitat for other delphinids (Dahood, 2009; Ingram and Rogan, 2002). Depth sensors were present on a limited number of tags in this study, and suggest that resident killer whales in this area regularly dive to or near the seafloor in 200–300 m (Matkin et al., 2013). Chum salmon in Japan have been observed to dive to the bottom in response to presence of Dall's porpoise (*Phocoenoides dalli*) (Yano et al., 1984), and Chinook salmon have been documented diving 300–400 m after release (Candy and Quinn, 1999). Furthermore, DTAGs deployed on northern resident killer whales in British Columbia documented the capture of Chinook, chum, and coho salmon as deep as 264, 164, and 165 m respectively (Wright, 2014). If salmon aggregate in these deep basins near the entrances to avoid predation, or to feed on congregating forage fish such as herring, the use of deeper waters within Montague Strait and near Kayak Island could be explained. Interestingly, many other deep glacial trenches in the continental shelf do not appear to be important for these Gulf of Alaska resident killer whales during the summer and fall.

Alternatively, the deep waters of Montague Strait, Hinchinbrook Entrance, and Kayak Island could provide important foraging opportunities on benthic species, including Pacific halibut (*Hippoglossus stenolepis*), lingcod (*Ophiodon elongatus*), and sablefish (*Anaplopoma fimbria*). It would be unlikely to be able to collect tissue samples from predation events on these species at the surface if they were consumed in deeper waters. However, despite the availability of these potential prey species in other deep waters at the edge of the continental shelf and in the deep glacial trenches that cut across the shelf, these locations were not used much by tagged individuals in this study. Additionally, recent studies of killer whale fecal samples from the southern resident killer whale population in the San Juan Islands demonstrate similar findings to the surface collections of fish scale and tissue after predation events, which is that salmonid prey dominate the diet in summer months (Ford et al., 2016). The seasonality of use by killer whales in Montague Strait, Hinchinbrook Entrance, and Kayak Island also supports surface observations of salmon predation (Matkin et al., 2013; Saulitis et al., 2000).

One of the important revelations of this project, and one of the main advantages of satellite telemetry over other methods of space use assessment, was the discovery of previously unknown core use areas. The region just west of Kayak Island appears to be an important area, particularly in June, July, and August (Fig. 2). Additionally, the areas southeast of Marmot Island and northeast of Shuyak Island appear to be important for at least the AD5 and AY pods (Fig. 1). Due to the remote location and difficult weather conditions, these areas would not likely be revealed by boat surveys, which can be biased by survey effort (Baird et al., 2010). Interestingly, most of the use near Kayak Island was from AB, AI, and AJ pods, and nearly every tagged member of AB, AI, and AJ pods visited this area. In the future, passive acoustics may help detail the importance of these areas.

The strong temporal patterns and pod-specific core use described in the present study should be considered in conservation management strategies. As an example, vessel traffic in the oil tanker lanes through

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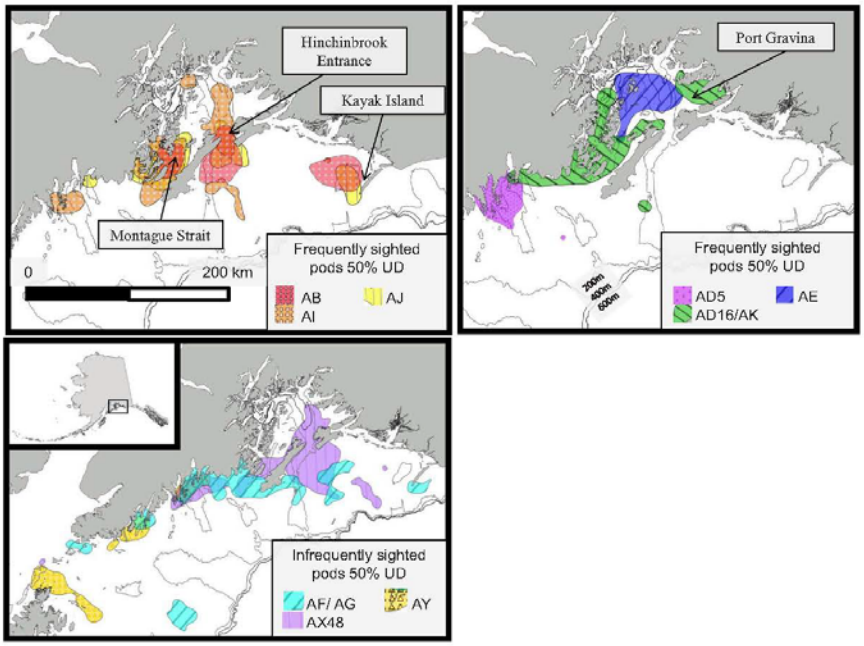

Fig. 4. Pod-specific variation in space use for resident killer whales (*Orcinus orca*). Pod 50% UD is displayed with color and symbols; AB (red with triangles), AI (orange with circles), AJ (yellow with vertical lines), AD5 (violet with circles), AD16/AK (green with back diagonal), AE (blue with forward diagonal), AF/AG (light blue with forward diagonal lines), AX48 (violet with vertical lines), AY (gold with triangles). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Hinchinbrook Entrance may have a much larger impact on resident killer whales in June and July than in September and October, and impact by winter vessel traffic is largely unknown. Additionally, the AB pod, which lost 25% of its members after swimming through the Exxon Valdez Oil Spill in 1989 (Matkin et al., 2008), appears to depend heavily on Hinchinbrook Entrance, Montague Strait, and the waters west of Kayak Island. Restoration plans for the AB pod should consider the protection of these areas. Future research should investigate the relationship between seasonal differences in core use and salmon migration routes, and also examine wintertime use.

From this study we have two main conclusions. First, is that core use areas in this population have extremely high variability between pods,

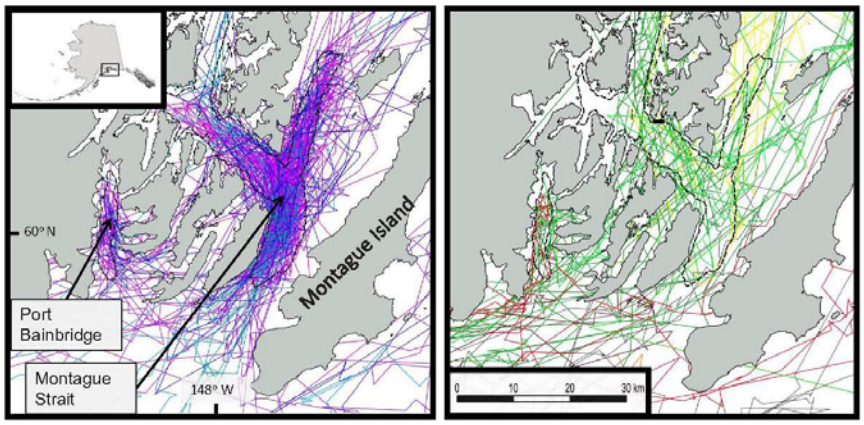

Fig. 5. Disproportionate use in lower Montague Strait and Port Bainbridge, with 200 m bathymetric contour (black dashed line). AB (blue), AI (light blue), and AJ pods (violet) are displayed together on the left panel. AD5 (gold), AD16/AK (green), AE (yellow), AF/AG (black), AX48 and AY pods (dark red), are displayed on the right panel. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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which may be due to cultural transmission within matrilineal groups. The second is that there are distinct seasonal differences in use patterns. These differences may be in response to the migratory return and feeding congregations of various species of salmon. Continued diet studies are warranted to investigate relationships between these seasonal differences in space use and the seasonal abundance of available prey.

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Appendix 2
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Geographic Patterns of Genetic Differentiation among Killer Whales in the Northern North Pacific

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Abstract

The difficulties associated with detecting population boundaries have long constrained the conservation and management of highly mobile, wide-ranging marine species, such as killer whales (*Orcinus orca*). In this study, we use data from 26 nuclear microsatellite loci and mitochondrial DNA sequences (988 bp) to test a priori hypotheses about population subdivisions generated from a decade of killer whale surveys across the northern North Pacific. A total of 462 remote skin biopsies were collected from wild killer whales primarily between 2001 and 2010 from the northern Gulf of Alaska to the Sea of Okhotsk, representing both the piscivorous "resident" and the mammal-eating "transient" (or Bigg's) killer whales. Divergence of the 2 ecotypes was supported by both mtDNA and microsatellites. Geographic patterns of genetic differentiation were supported by significant regions of genetic discontinuity, providing evidence of population structuring within both ecotypes and corroborating direct observations of restricted movements of individual whales. In the Aleutian Islands (Alaska), subpopulations, or groups with significantly different mtDNA and microsatellite allele frequencies, were largely delimited by major oceanographic boundaries for resident killer whales. Although Amchitka Pass represented a major subdivision for transient killer whales between the central and western Aleutian Islands, several smaller subpopulations were evident throughout the eastern Aleutians and Bering Sea. Support for seasonally sympatric transient subpopulations around Unimak Island suggests isolating mechanisms other than geographic distance within this highly mobile top predator.

Key words: *ecotypes, genetic structure, mtDNA, microsatellite, Orcinus orca, populations, subpopulations*

Population boundaries are often difficult to define for highly mobile species with largely continuous geographical distributions. However, identifying patterns of population structure is critical for the effective management and conservation of natural populations, and for identifying subpopulations requiring unique management strategies (Avise 1994). Furthermore, underlying population genetic structure has considerable evolutionary and ecological relevance, providing unique insight into mechanisms of reproductive isolation and patterns of localized adaptation, and furthering our understanding of the factors that shape these subdivisions and drive divergence. Beyond population delimitation and identification of stock boundaries, understanding patterns of gene flow and dispersal is fundamental for evaluating population status.

High mobility and dispersal capabilities, combined with a seemingly homogenous marine habitat, were initially assumed to translate into high levels of gene flow within oceanic species (Palumbi 1994). Analytical advances have provided the tools necessary to directly examine geographic structuring among individual animals, and recent studies of a variety of marine vertebrate species have clearly demonstrated that high

potential mobility cannot be used as a predictor of effective gene flow (Carreras et al. 2007; Verissimo et al. 2010; Sandoval-Castillo and Rocha-Olivares 2011). Despite the lack of obvious physical barriers to dispersal and gene flow, molecular genetic studies of many species within the taxonomic order Cetacea have clearly dispelled the assumption of panmixia, documenting numerous cases involving significant geographic patterns of population genetic differentiation (Baker et al. 1998; Rosel et al. 1999; Parsons et al. 2006; Fontaine et al. 2007; Mirimin et al. 2009; Rosenbaum et al. 2009). Because cetaceans are marine predators with remarkable longevity and both direct and indirect interactions with commercial fisheries, understanding the structuring of their populations has important implications for understanding ecosystem processes on both local and global scales.

The killer whale (*Orcinus orca*), a large, globally distributed delphinid, is among the better known of cetacean species. In the northeastern Pacific, long-term studies on several small populations of piscivorous killer whales have contributed unprecedented insight into their habits, social organization, philopatry to matrilineal groups and, more recently, patterns of gene flow (Balcomb and Bigg 1986; Bigg et al. 1990; Parsons et al. 2009; Ford et al. 2011). Studies focusing on the behavioral ecology of killer whales have identified 3 divergent yet sympatric ecotypes inhabiting northern North Pacific waters (Bigg 1982; Ford et al. 1998). The 3 ecotypes (commonly referred to as "residents," "transients," or Bigg's killer whales, in tribute to the late Dr Michael Bigg (Ford 2011; Riesch et al. 2012), and "offshores") differ phenotypically and show marked differences in patterns of dispersal, acoustic patterns, social structure, group dynamics, and prey preferences (Baird and Stacey 1988; Bigg et al. 1990; Ford 1991; Barrett-Lennard et al. 1996; Ford et al. 1998; Baird and Whitehead 2000; Foote and Nystuen 2008; Ford et al. 2011). In addition to the genetic differences among ecotypes first described by Stevens et al. (1989) and Hoelzel and Dover (1991), recent analyses of the entire mitochondrial genome suggested that some of the unique killer whale ecotypes represent deeply divergent evolutionary lineages and warrant elevation to species or subspecies status (Morin et al. 2010). For example, estimates from mitogenome sequence data indicate that transient killer whales diverged from all other killer whale lineages some 700 000 years ago, and the ad hoc committee on marine mammal taxonomy currently recognizes the 2 predominant North Pacific ecotypes as unnamed *Orcinus orca* subspecies (Committee on Taxonomy 2012). Coalescent analyses further suggest that the ecological divergence between the resident and transient ecotypes may have arisen during an allopatric period preceding the migration of ancestral resident maternal lineages back into the North Pacific resulting in secondary contact and the current sympatric distribution (Foote et al. 2011). The broad distribution of killer whales throughout coastal and offshore waters, combined with its ecological specializations, presents an ideal opportunity to compare patterns of genetic structuring among ecotypes and contrast the socioecological factors that shape patterns of gene flow and population structuring.

As a result of multiple decades of individual-based studies, population structure is well characterized for killer whales around Prince William Sound/Kenai Fjords, in the coastal waters of the Gulf of Alaska (Matkin 1997; Matkin et al. 1999), and for those inhabiting the coastal waters further south around British Columbia and Washington State (Bigg et al. 1990; Ford 1991; Baird and Whitehead 2000; Ford et al. 2011). However, less information is available for whales inhabiting waters of the western Gulf of Alaska, Aleutian Islands, Bering Sea, and Russia. Despite a relatively ubiquitous distribution, data documenting individual movements and social affiliations (Durban et al. 2010; Fearnbach 2012), as well as telemetry data (Durban J, unpublished data; Matkin et al. 2012) suggest that some individuals and matrilineal pods exhibit restricted movements and a high degree of interannual site fidelity. However, contemporary estimates of gene flow are lacking for these northern areas, and documented movements of individual whales between Kodiak Island and southeastern Alaska, for example, suggest a certain degree of connectedness (Matkin 1997; Matkin et al. 1999, 2012). As a consequence of the uncertainty surrounding the population structuring within these regions and a lack of data for the westernmost reaches of the northern North Pacific, current stock designations encompass very broad areas. According to the stock assessment requirements of the US Marine Mammal Protection Act (MMPA), resident killer whales inhabiting the waters in the far North Pacific are currently recognized as a single stock ranging from southeast Alaska through the Aleutian Islands and Bering Sea (Allen and Angliss 2011). The US MMPA stock designation for transient killer whales recognizes 2 stocks with overlapping geographic distributions, comprising the "Aleutian and western" stock (Gulf of Alaska, Aleutian Islands, and Bering Sea), and the much smaller community of "AT1" killer whales whose range appears to be largely restricted to Prince William Sound and the Kenai Fjords (Allen and Angliss 2011; Matkin et al. 1999). Recent work examining the social structure of resident killer whales within the "Alaska resident stock" described social networks that are spatially connected yet exhibit differential ranging patterns (Fearnbach 2012). Such socially mediated spatial structuring may provide a basis for population genetic subdivisions similar to that described for the Northern and Southern resident killer whale communities off the coast of British Columbia and Washington State (Ford et al. 2000).

As apex predators with high energetic requirements (Noren 2011; Williams et al. 2004, 2011), killer whales are of both management and conservation concern throughout the North Pacific. Predation on, and competition with, both endangered and commercially important species (e.g., marine mammals, salmonids) make killer whales a species of interest throughout Alaskan waters and beyond. In this study, we use both mitochondrial (mtDNA) sequences and nuclear (nDNA) microsatellite genotypes to examine genetic structure of 2 ecotypes (residents and transients) within the genus *Orcinus* in northern North Pacific waters. The patterns of genetic discontinuities resolved in this study will provide data to support a revision of stock structure in the North

Pacific and provide insight into some of the ecological factors shaping killer whale populations.

Methods

DNA Extraction and PCR Amplification

Skin biopsy samples were obtained from killer whales by remote dart biopsy (Barrett-Lennard et al. 1996; Parsons et al. 2003) during dedicated and opportunistic shipboard surveys across the North Pacific. Samples were collected primarily during the summer months (June through August), primarily between 2001 and 2010 from both resident and transient killer whales (Table 1). Tissue samples were stored frozen in 99% ethanol or salt-saturated dimethyl sulfoxide solution until the time of sample processing. Total genomic DNA was isolated from skin biopsy subsamples using a variety of common extraction methods, including silica-based filter membranes (Qiagen, Valencia, CA), standard phenol/chloroform extraction (modified from Sambrook et al. 1989), and lithium chloride (Gemmell and Akiyama 1996). DNA concentrations were determined by absorbance on a NanoDrop ND-8000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE) and normalized to a working concentration of 2 ng/μL. Remaining skin biopsy fragments and extracted DNA were archived at −80 °C.

The mitochondrial control region was amplified via polymerase chain reaction (PCR) in 20 μL reaction volumes as described in Zerbini et al. (2007). Both strands of the amplicon were sequenced independently using Applied Biosystems (ABI, Carlsbad, CA) BigDye Terminator v3.1 Cycle Sequencing Kit on the ABI model 3100 sequencer. Sequences were manually checked for sequencing errors or questionable base calls and aligned using ClustalW (Thompson et al. 1994) as implemented in BioEdit (Hall 1999). Control region haplotypes were assigned based on comparison with previously published killer whale sequences deposited in GenBank. Haplotypic (h) and nucleotide (π) diversities were estimated according to Nei (1987) to describe the control region sequence divergence and haplotype frequency differences using Arlequin v3.11 (Excoffier et al. 2005).

Samples were genotyped at 27 polymorphic microsatellite loci (see Supplementary Appendix 1 online). Initially, each locus was amplified individually in 10 μL reactions containing 4 ng of genomic DNA, 1X Promega GoTaq Flexi Buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.1 μg/μL of bovine serum albumin, 0.2 μM of each primer (forward primers were fluorescently labeled), and 0.5 units of GoTaq Flexi DNA Polymerase (Promega, Madison, WI). Thermocycler profiles included initial denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C for 35 s, T_a °C for 35 s, 72 °C for 35 s, and a final extension at 72 °C for 30 min. Amplification conditions were further optimized, and the majority of loci were multiplexed as groups of 2–4 loci with nonoverlapping allele sizes using the Qiagen Multiplex PCR Kit. Each multiplex PCR was performed according to the conditions suggested by Qiagen Multiplex PCR Kit handbook in a total reaction volume of 20 μL. Additional PCR conditions are described in Supplementary Appendix 1 online. Amplified products were analyzed using an ABI 3100 automated DNA sequencer, and allele sizes were determined using ABI LIZ500 as the internal size standard. ABI GeneScan v3.7 and Genotyper v3.7 (ABI) software were used to collect and analyze microsatellite data.

Genotyping quality control measures included negative control reactions at each step including DNA extraction, PCR, and sequencing, as well as replicate genotyping of multiple samples. An overall genotyping replication rate of ≥11% of samples allowed us to empirically estimate the per-allele genotyping error rate (Hoffman and Amos 2005; Morin et al. 2010). Furthermore, each PCR set included at least 2 samples previously genotyped to provide cross-plate controls and ensure consistent allele binning throughout the study.

Table 1 Sample sizes across a priori strata for both resident and transient killer whales sampled across the northern North Pacific

Geographic region	Ecotype: a priori stratum	Collection years	Number of samples		
			Total	Resident	Transient
Central Aleutians	RES-CAL	2001–2010	61	61	
Eastern Aleutians	RES-EAL	1997–2010	56	56	
Gulf of Alaska	RES-GOA	2001–2005	32	32	
Russia	RES-RUS	1994–2006	117	117	
Western Aleutians	RES-WAL	2004–2010	8	8	
Eastern Aleutians	TRANS-EAL	1990–2009	44		44
Unimak Island	TRANS-UI	2001–2009	16		16
Gulf of Alaska	TRANS-GOA	2004	13		13
Kamchatka Peninsula	TRANS-KAM	2002–2006	11		11
Kodiak Island	TRANS-KOD	2001–2005	7		7
Sea of Okhotsk	TRANS-OKH	2001–2004	6		6
Pribilof Islands	TRANS-PRI	2005–2009	30		30
Rat Island Group	TRANS-RAT	2006–2010	11		11
Tanaga Island	TRANS-TAN	2003–2010	5		5

Counts reflect the number of individually genotyped whales after the removal of genetically identical biopsies.

Ecotype Identification and Genetic Assignment

Ecotype identification for each sample was based on both photographic identification of individuals using phenotypically distinctive characteristics of whales in sampled groups and mitochondrial control region sequence (Matkin et al. 2007; Zerbini et al. 2007; Durban et al. 2010). The ability to reliably identify ecotype based on characteristic pigmentation and morphological differences (Baird and Stacey 1988; Ford et al. 2000) and fixed mtDNA sequence differences (Hoelzel et al. 1998; Barrett-Lennard 2000; Hoelzel et al. 2002) has been previously demonstrated for North Pacific killer whales (Zerbini et al. 2007). For the 6 samples in the data set for which the above data were unavailable, ecotype was identified post hoc by examining the clustering of samples in a principal coordinate analysis (PCA) based on multilocus data and by individual assignment tests as executed in GeneClass (see below).

The probability of an individual belonging to a particular ecotype was estimated using the Bayesian assignment method of Rannala and Mountain (1997) as implemented in GeneClass v.2 (Piry et al. 2004), and the clustering algorithms implemented in STRUCTURE (Pritchard et al. 2000), run naively without the inclusion of prior information on ecotype or location (see below for model specifics). The clustering of individual samples according to pairwise genotypic distance was examined using PCA as implemented in GenAlEx v.6.4 (Peakall and Smouse 2006). Genetic differentiation (nDNA) between resident and transient ecotypes was estimated using both F_{ST} (Weir and Cockerham 1984) and F'_{ST} (Hedrick 2005), calculated using custom code (Mesnick et al. 2011) written in the statistical programming language R (R Development Core Team 2011). Arlequin v.3.5.1.2 (Excoffier and Lischer 2010) was used to estimate both F_{ST} and Φ_{ST} (Tamura and Nei, 1993; $\alpha = 0.5$) for mtDNA sequence data. Statistical significance for all metrics was determined by 10 000 random permutations of the original data set.

Identifying Duplicate Samples, Estimating Genetic Diversity, and the Removal of Close Kin

Microsatellite Toolkit (Park 2001) and GENECAP (Wilberg and Dreher 2004) were used to examine the microsatellite genotype data set for potential errors and to identify duplicate genotypes by comparing each multilocus genotype to all others in the data set. All pairs of genotypes that mismatched at 3 or fewer loci were rechecked for potential scoring errors by re-examining the electropherograms for those loci. Pairs of samples that were identified as genetic matches were further examined by comparing associated field (photographic identifications) and molecular (control region haplotypes and genetic sex) data. GENECAP (Wilberg and Dreher 2004) was also used to calculate the probability of identity ($P_{(ID)}$): the probability that 2 unrelated individuals share the same multilocus genotype by chance. The observed $P_{(ID)}$ was calculated using the more traditional formula assuming Hardy–Weinberg equilibrium (HWE; Paetkau and Strobeck 1994), as well as the conservative estimator of $P_{(ID)}$ for full siblings ($P_{(ID)sib}$; Waits et al. 2001). Estimates for $P_{(ID)sib}$ were used to empirically assess a minimum threshold for the number of loci genotyped by calculating $P_{(ID)sib}$ for increasing numbers of loci. Including data from the least heterozygous loci first, we derived a conservative estimate of the minimum number of loci needed to identify individual whales and achieve a probability of identity for siblings $\leq$0.001 (Waits et al. 2001).

After removal of duplicate samples from the data set, genetic diversity within each ecotype was quantified as the mean number of alleles per locus (Na), allelic richness (AR), observed (H_O) and expected heterozygosity (H_E), and inbreeding coefficient (F_{IS}) using FSTAT (Goudet 2000) and GenAlEx (Peakall and Smouse 2006). Departures from HWE expectations using the Fisher's Exact test (Guo and Thompson 1992) and tests for genotypic disequilibrium among the loci were assessed using GENEPOP v4.0 (Raymond and Rousset 1995). Multiple tests error rate was adjusted using the sequential Bonferroni correction (Rice 1989).

Data sets containing a large number of closely related individuals have the potential to impact estimates of population structure and inflate measures of genetic distance through violations of model assumptions due to allelic enrichment (Amos et al. 1993). Long-term studies of several killer whale populations have documented extreme philopatry to natal groups and a matrifocal social organization within populations (Balcomb and Bigg 1986; Bigg et al. 1990; Ford et al. 1994 Matkin et al. 1999; Parsons et al. 2009). Because the focus of this study is to examine population structure on a fairly broad scale geographically, we addressed potential kin bias by estimating pairwise relatedness within each ecotype from microsatellite allele frequency data. KINGROUP (Konovalov et al. 2004) was used to estimate pairwise relatedness according to Lynch and Ritland's (1999) regression-based estimator (R_{LR}). Relatedness estimates were compared with the maximum value obtained from a simulated set of 10 000 pairs of unrelated individuals (UR) using the observed allele frequencies. Pairs of individuals with $R_{LR} > UR_{MAX}$ were considered to be potential close relatives and 1 individual from the pair was removed for analyses of spatial genetic patterns to minimize the impact of inclusion of kin in the data set.

Testing a priori Hypotheses of Geographic Structure

Geographic structure was first examined by testing a priori subdivisions. Putative geographic strata were defined based on data acquired from georeferenced photographic records of individual killer whales (Wade P, Durban J, unpublished data; Durban et al. 2010), the geographic extent of social network clusters (Fearnbach 2012), and the presence of large geophysical barriers (e.g., Kamchatka peninsula). Strata names were based on general geographic regions, and samples were assigned to the stratum in which they were sampled. These assignments are not intended to convey core areas for individually sampled killer whales. Despite some long-range movements of individual whales, social network analyses highlight a strong spatial component that was used to inform a priori strata (Fearnbach 2012). However, individual sighting histories were limited for the majority of killer whales encountered in the Aleutian Islands and Bering

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Sea. Therefore, with the exception of transient killer whales comprising the Unimak Island stratum (see below), spatial genetic structure was tested by assigning individual whales to the stratum in which they were sampled.

Resident killer whales were assigned to 5 large a priori subdivisions delimiting putative populations that were arranged largely along longitudinal lines and significant oceanographic boundaries in the North Pacific (Figure 1a): Russia (RUS), western Aleutian Islands (WAL), central Aleutians (CAL), eastern Aleutians (EAL), and the Gulf of Alaska (GOA). Transient killer whales were assigned to 9 smaller putative subdivisions: Sea of Okhotsk (OKH), Kamchatka peninsula (KAM), the Rat Islands group (RAT), Tanaga Island (TAN), Pribilof Islands (PRI), eastern Aleutian Islands (EAL), Unimak Island (UI), Kodiak Island (KOD), and the Gulf of Alaska (GOA) (Figure 2a). In the eastern Aleutians, samples were assigned to the Unimak Island (UI) stratum based on behavioral data documenting the presence of identified whales in spring killer whale assemblages foraging on migrating gray whales (Barrett-Lennard et al. 2011; Durban et al. 2010). A priori hypotheses about population structure were first tested by estimating both nuclear and mitochondrial genetic differentiation among these strata. Measures of genetic differentiation including pairwise measures of F_{ST} (Weir and Cockerham 1984), F'_{ST} (Hedrick 2005), G'_{ST} (Hedrick 2005; Meirmans and Hedrick 2010) and chi square were calculated from nuclear microsatellite data using the custom R code as described above. Both F_{ST} and

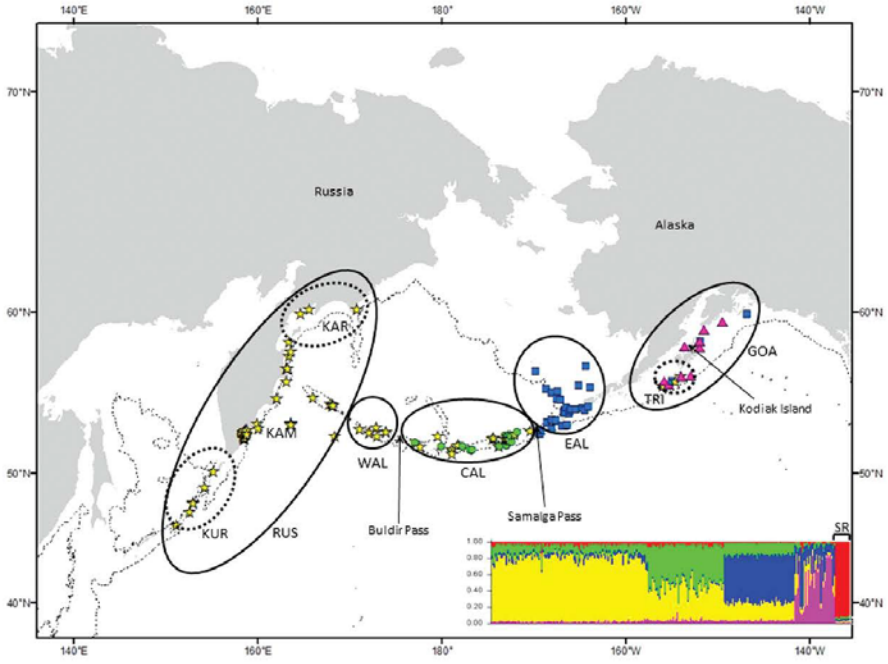

Figure 1. Resident killer whale samples included in this study plotted according to biopsy sample locations. **(a)** Solid line ellipses indicate the extent of a priori geographic strata. Dotted lines surround putative strata indicated by Wombling analyses and included in pairwise tests of genetic differentiation. Symbols representing individual samples are colored according to the STRUCTURE cluster (model for $k = 5$) to which they were assigned with the highest probability (mean ± SD = 0.677 ± 0.143). Inset figure shows the STRUCTURE bar plot ($k = 5$), where each vertical bar represents the proportional membership of individual whales within each of inferred genetic clusters, individuals are ordered by longitude. Samples representing the southern resident killer whale population ("SR" on the far right of the inset plot) sampled in Washington State are not mapped. **(b)** Ellipses indicate a posteriori geographic strata based on analysis of nDNA and mtDNA data. Individual samples are coded according to control region mtDNA haplotype. Inset figure shows regions of genetic discontinuity (light grey) identified by WOMBSOFT indicating significant putative genetic boundaries for resident killer whales. The 1000 m bathymetric depth contour is indicated by a thin broken line.

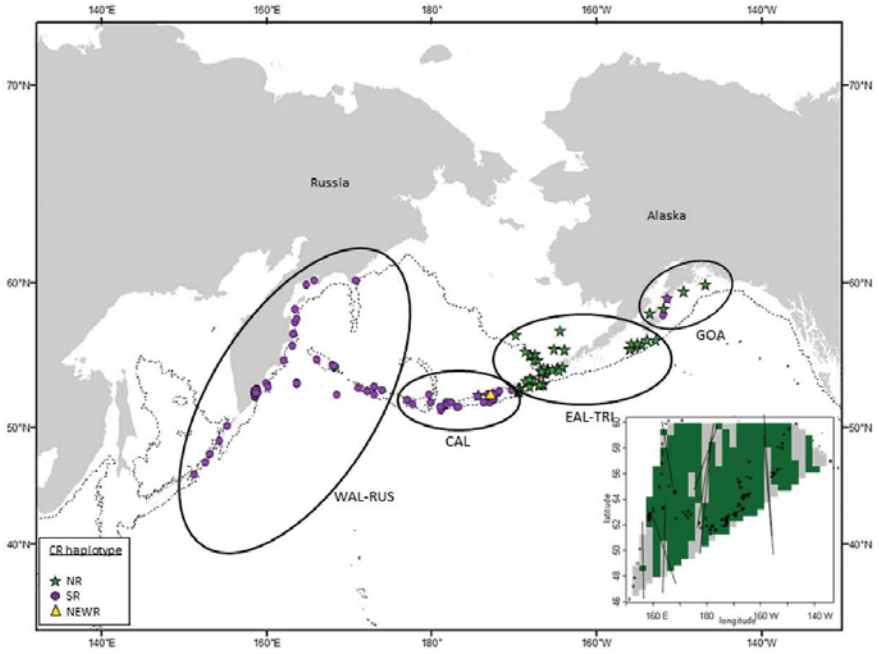

Figure 1. Continued

Φ_{ST} overall, and for all pairwise comparisons among strata, were estimated as above for mtDNA sequence data.

Detecting Spatial Genetic Clusters

The presence of spatial genetic discontinuities or population boundaries that were not reflected by the a priori subdivisions was explored using 2 complementary methods. First, the Wombling method was applied as implemented in the R package, WOMBSOFT (Crida and Manel 2007). This method uses geographically referenced individual genotypes to compute allele frequencies across the study region, and calculates the gradient of these surfaces to infer genetic boundaries between populations (Zhu et al. 2011). Default values were used for the WOMBSOFT models, with the exception of the grid size that was set at 30 × 30 across the entire study area and a bandwidth of $b = 1.0$. Longitudes were manually transformed to avoid negative values east of 180°, facilitating interpretation of the resulting candidate boundaries map. Statistical significance of genetic boundaries was assessed at a level of $\alpha = 0.05$.

The Bayesian clustering algorithm implemented in STRUCTURE 2.3 (Pritchard et al. 2000) was used to estimate the number of genetically distinct subpopulations, assuming the admixture model with correlated allele frequencies. Although photographic evidence suggests population subdivisions, repeated sightings of killer whales throughout the Aleutian Islands indicate infrequent movement between neighboring geographic strata (National Marine Mammal Laboratory, unpublished data; Durban et al. 2010). In light of these movements and the generally weak signals of population genetic structure resolved for other cetacean populations, it is reasonable to expect relatively weak signals of genetic differentiation. As such, we applied the new models of Hubisz et al. (2009), incorporating general sample locations to inform cluster assignments, rather than the original STRUCTURE model of Pritchard et al. (2000) that incorporates prior information based on the existence of relatively well-supported discrete populations. The sampling location prior (LOCPRIOR) was assigned according to the a priori geographic strata described above. STRUCTURE was run independently both with and without the sampling location prior. We executed 5 independent runs of 10^5 iterations (after burn-in of 10^5 iterations) for each model to estimate the probability support for each number of candidate clusters, k, from 1 to 20. The most likely number of clusters, k, was determined by the method of Pritchard et al. (2000). We also estimated the statistic Δk that quantifies the second-order rate of change in log-likelihood across the range of k values as described by

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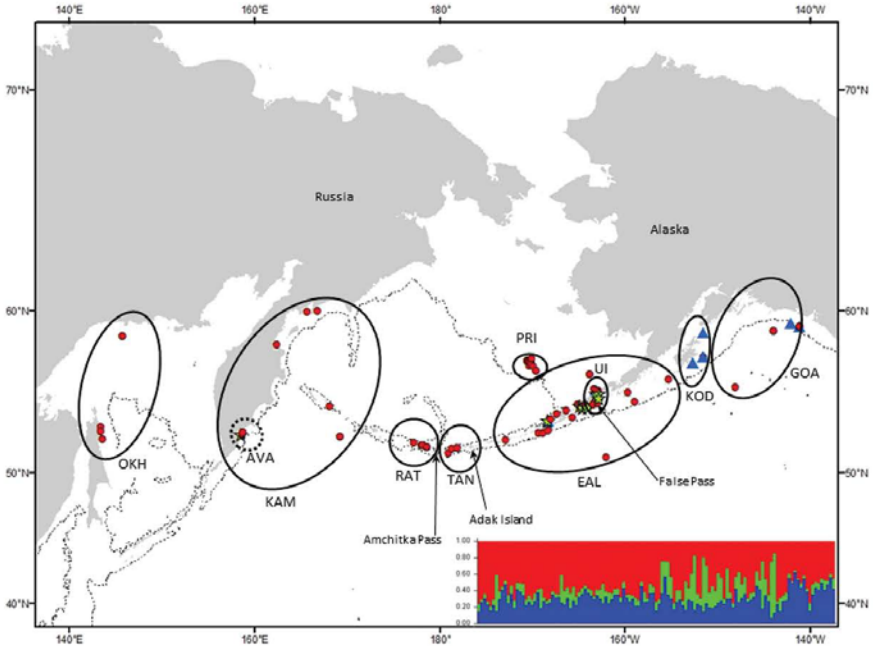

Figure 2. Transient killer whale samples included in this study plotted according to biopsy sample locations. **(a)** Solid line ellipses indicate the extent of a priori geographic strata. Dotted lines surround a putative stratum indicated by Wombling analyses and included in pairwise tests of genetic differentiation. Symbols representing individual samples are colored according to the STRUCTURE cluster (model for $k = 3$) to which they were assigned with the highest probability (mean ± SD = 0.591 ± 0.100). Inset figure shows the STRUCTURE bar plot ($k = 3$), where each vertical bar represents the proportional membership of individual whales within each of inferred genetic clusters, individuals are ordered by longitude. **(b)** Ellipses indicate a posteriori geographic strata based on analysis of nDNA and mtDNA data. Individual samples are coded according to control region mtDNA haplotype. Inset figure shows regions of genetic discontinuity (light grey) identified by WOMBSOFT indicating significant putative genetic boundaries for transient killer whales. The 1000 m bathymetric depth contour is indicated by a thin broken line.

Evanno et al. (2005) and directly examined STRUCTURE bar plots for likely values of k.

Genetic cluster analyses were performed for the 2 ecotypes separately, acknowledging the recent findings of mitogenomic analyses that indicated high levels of genetic divergence suggesting that these 2 North Pacific ecotypes may in fact represent separate species (Foote et al. 2011; Morin et al. 2010). In addition to samples collected in the northern North Pacific, STRUCTURE analysis of the resident killer whale data set included a subset of whales ($n = 11$) from the southern resident killer whale (SRKW) population. Despite the relatively continuous distribution of resident killer whales along the west coast of North America, a number of genetically and demographically distinct populations are currently recognized. The SRKW population is recognized as a distinct population segment inhabiting the waters between British Columbia and northern California and is both geographically segregated and genetically distinct from the Alaskan populations (Barrett-Lennard 2000; Ford et al. 2000; Krahn et al. 2004; Ford et al. 2011). Furthermore, recent genetic analyses found no evidence to suggest that calves were sired by males outside the population, further supporting a lack of gene flow between the SRKW population and neighboring populations (Ford et al. 2011). This subset of SRKW samples was included to provide an independent method for assessing the model's ability to identify this set of samples as a unique genetic cluster.

Quantifying Genetic Differentiation among Subpopulations

Patterns of genetic differentiation among a priori strata were examined for each ecotype using microsatellite genotypes

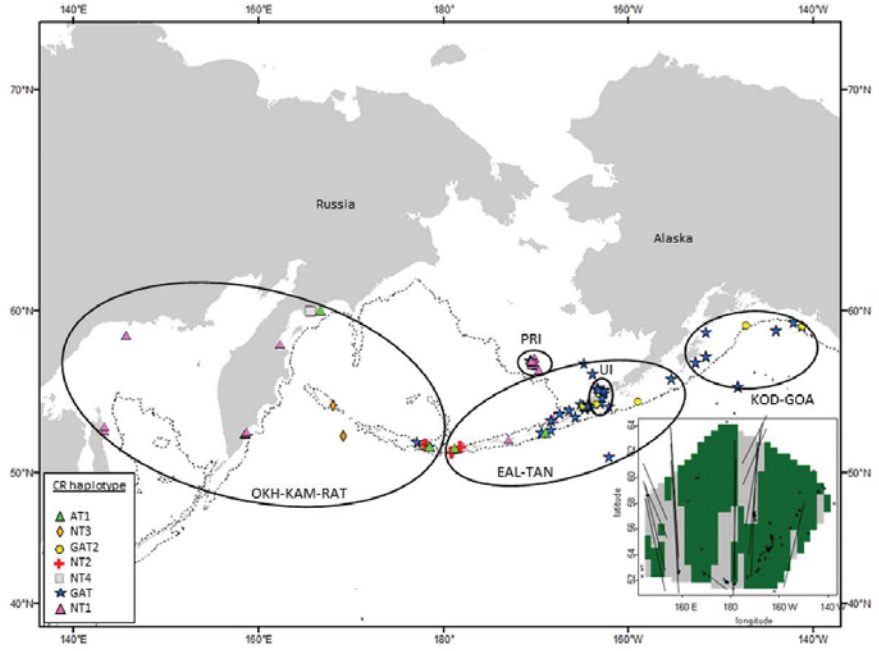

Figure 2. Continued

and mtDNA sequences. Lack of statistical support for geographically neighboring strata was taken as evidence of larger geographic population strata, and putative population boundaries were redrawn accordingly. The spatial extent of genetic clusters inferred from the results of both WOMBSOFT and STRUCTURE were compared with a priori strata. Where the spatial genetic models suggested regions of significant genetic differentiation not reflected by the original a priori subdivisions, new boundaries were drawn a posteriori, and pairwise measures of genetic differentiation among these secondary putative strata were recalculated as described above for both mtDNA and nDNA to provide quantitative metrics for comparison.

Results

Genetic Diversity and Ecotype Differentiation

Molecular genetic analyses were applied to 462 killer whale biopsy samples collected throughout the study range between the northern Gulf of Alaska and the Sea of Okhotsk (Table 1; Figures 1a and 2a; see Supplementary Appendix 2 online). Ecotype was determined for each sample on the basis of photographic (phenotypic) evidence and mtDNA control region haplotype for 98.67% of samples. The absence of discrepancies between the mtDNA data and photographic-based ecotype assignments clearly supports the validity of these 2 independent methods for ecotype determination and corroborates previous findings for North Pacific killer whales (Durban et al. 2010; Matkin et al. 2007; Zerbini et al. 2007).

Ten unique haplotypes (Table 2) were defined based on nucleotide differences across the mitochondrial control region (~988 bp). Both haplotypic and nucleotide diversity were low, consistent with expectations considering previously published studies of killer whale mitochondrial diversity (Table 3; Hoelzel et al. 2002; Zerbini et al. 2007; Morin et al. 2010). Seven mtDNA haplotypes were detected from transient killer whale samples ($n = 153$), whereas only 3 haplotypes were represented among the resident killer whale samples ($n = 288$), with one of these (NEWR) found in only a single whale. No mtDNA haplotypes were shared between the 2 ecotypes. The geographic distribution of the 2 common resident haplotypes was strongly differentiated by a break ($F_{ST} = 0.898$, $P < 0.0001$; $\Phi_{ST} = 0.915$, $P < 0.0001$) at Samalga Pass (170°W), delimiting the western domination by NR and the eastern domination by the SR haplotypes (Figure 1b). Only 5 samples with the NR haplotype were found west of Samalga Pass, but both haplotypes co-occurred in the GOA east of KOD (153°W). In contrast, the distribution of control region haplotypes for transient killer whales was much less

Table 2 Control region (mtDNA) haplotype identity and frequency across sampled resident and transient killer whales for which high quality sequences were generated (n = 405)

GenBank accession number	Variable sites	Ecotype	Frequency	Common names
	122234444457 267980499938 475530934637			
DQ399077	TGTATACACCTA	Resident	176	SR, ENPSR
DQ399078	.........T..	Resident	86	NR, ENPNR
DQ399074	..C.........	Resident	1	NEWR
DQ399082	...GC.T.T.CG	Transient	17	AT1
DQ399081	....CGT.T.CG	Transient	68	GAT
DQ399080	.....GT.T.CG	Transient	11	GAT2, ENPT2
DQ399075	.A..CGT.T.C.	Transient	35	NT1
DQ399076	C...CGT.T.CG	Transient	6	NT2
GU187157	..CGC.T.T.CG	Transient	3	NT3
GU187161	.A..CGTGT.C.	Transient	2	NT4

Variable nucleotide sites within the 980 bp mtDNA fragment are indicated.

discrete (Figure 2b) although differences in the frequency of occurrence were evident across the region.

All 27 microsatellite loci were polymorphic. The number of alleles per locus ranged from 3 (Ttr04) to 12 (EV37*Mn*), with an average of 7.22 alleles per locus (see Supplementary Appendix 1 online). Evidence of private alleles was found for both resident and transient ecotypes (Table 3). In general, genetic diversity was higher among transient killer whales (Table 3). The average rate of missing data per locus due to amplification errors was 11.11% (SD = 3.24%), excluding 10 samples that failed to amplify at all loci due to poor sample/DNA quality. Global tests for deviation from HWE within each ecotype revealed heterozygote deficiencies for 7 out of the 27 loci (EV5*Pm*, KW207, Dde66, 415/416, GATA53, 417/418, and FCB5). However, only KW207 showed evidence of significant departures from HWE for both ecotypes after correction for multiple tests. Plots of H_O/H_E (see Supplementary Appendix 3 online) for each locus confirmed an obvious heterozygote deficit for KW207, and this locus was subsequently dropped from all further analyses. No evidence of genotypic disequilibrium was detected among loci after correction for multiple tests.

Examination of multilocus genotypes for evidence of duplicate genotypes revealed multiple "recaptures" of 23 genotypes, including 21 duplicate and 2 triplicate samples. Original electropherograms were carefully reviewed for all putative matching genotypes mismatching at ≤3 loci. A per-allele genotyping error rate of 0.24% was empirically estimated from replicated positive control samples. The most conservative estimate of probability of identity ($P_{(ID)sib}$) was used to provide a lower bound on the number of loci required to reliably distinguish among even closely related individuals. Calculating $P_{(ID)sib}$ for an increasing number of loci, with increasing heterozygosity, indicated that a minimum of 10 loci were required to achieve a conservative $P_{(ID)sib}$ estimate of 0.00078. This probability of identity was used to identify genotypes of sufficient quality, and all samples typed at fewer than 10 loci were removed from subsequent analyses. After the removal of duplicate and triplicate genotypes, and samples typed at ≤10 loci, a total of 391 individuals (residents = 264; transients = 127) were included in all spatial genetic analyses.

Table 3 Measures of genetic diversity for both nuclear and mitochondrial loci

	Resident killer whales	Transient killer whales
mtDNA		
n	265	142
haplotypes	3	7
h	0.4503 ± 0.0198	0.6815 ± 0.0303
π	0.0005 ± 0.00046	0.0042 ± 0.0023
Microsatellite		
n	263	143
AR	3.647 (±0.917)	6.701 (±2.242)
N_A	4.000 (±1.095)	6.769 (±2.303)
H_O	0.441 (±0.145)	0.597 (±0.181)
H_E	0.479 (±0.153)	0.647 (±0.184)
F_{IS}	0.113 (±0.190)	0.075 (±0.076)
A_{PRI}	0.577 (±0.138)	3.346 (±0.363)

Values reflect the final data set of 26 microsatellite loci after the removal of duplicate genotypes and poor quality samples that failed across all loci.
h, haplotypic diversity; π nucleotide diversity; AR, allelic richness; NA, mean number of alleles; A_{PRI}, private alleles averaged across all loci.

PCA plots showed clear clustering of samples by ecotype (Figure 3), and 99.8% of samples correctly self-assigned to ecotype using GeneClass. The single sample that misassigned had a probability of assignment of 54% to the alternate population, but assigned to the correct population with a probability of 46%. This assignment ambiguity was likely attributed to missing data at 15 out of 26 loci. All 6 samples of unknown type were assigned to the resident ecotype with an average assignment value of 0.929 (±0.075), supporting the clustering observed in the PCA plot, and were therefore determined to originate from a resident killer whale population. In addition to the absence of shared mtDNA haplotypes

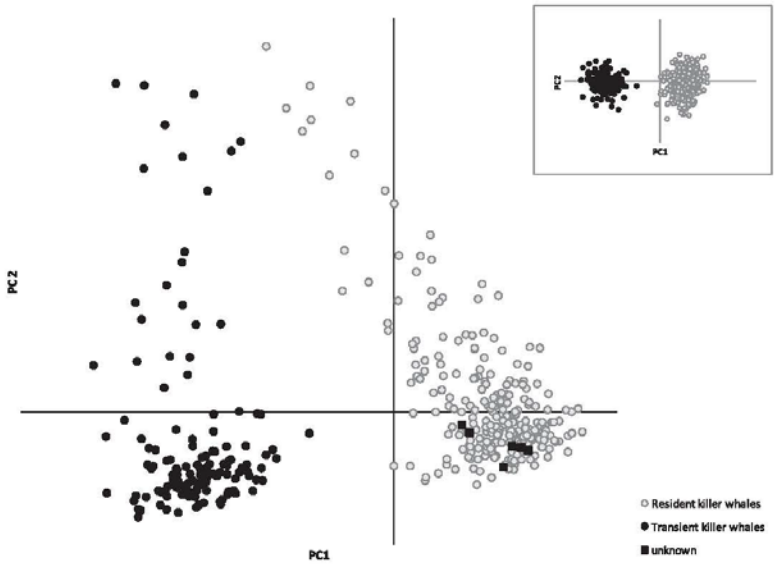

Figure 3. Plot of first 2 principal coordinates based on microsatellite data (26 loci) for all killer whale samples genotyped from the northern North Pacific. The long converging tails are an artifact of samples with incomplete genotypes and are eliminated when the data set is further restricted to samples genotyped at ≥20 loci (n = 372; inset figure).

(Table 2; $F_{ST} = 0.447$, $P < 0.0001$; $\Phi_{ST} = 0.865$, $P < 0.0001$), estimates of genetic distance indicated highly significant nDNA divergence between the 2 North Pacific killer whale ecotypes ($F_{ST} = 0.2104$, $F'_{ST} = 0.4690$, $P = 0.0001$). The deep genetic divergence between ecotypes was further supported by a cluster analysis performed in STRUCTURE, without prior information on ecotype or sampling location. The results grouped all North Pacific samples into one of two clusters, assigning individual samples with remarkable confidence (mean ± SD = 0.9950 ± 0.01502). All individual whales correctly assigned to one of two clusters comprised exclusively of either resident or transient killer whales.

Identification of Spatial Genetic Clusters

Relatedness estimates (R_{LR}) based on a simulated data set using the observed allele frequencies resulted in a maximum estimate of R_{LR} for unrelated pairs of individuals (UR_{MAX}) of 0.571 (mean ± SD = 0.001 ± 0.086) for transient killer whales, and $UR_{MAX} = 0.816$ (mean ± SD = 0.0007 ± 0.129) for resident killer whales. Using UR_{MAX} as a minimum threshold for estimates of relatedness between potential kin, 9 pairs of resident and 4 pairs of transient killer whales were identified as putative close relatives. One individual from each pair of putative relatives was removed from the data set. Subsequent data analyses were performed on the data representing only unrelated individuals, and all spatial genetic analyses were conducted separately for each ecotype.

Genetic Structure of Resident Killer Whales

Measures of genetic differentiation among the 5 putative a priori strata of resident killer whales showed significant mtDNA differentiation among all neighboring strata in the Aleutian Islands, and significant nDNA genetic differentiation among all pairwise comparisons except RUS and WAL (Table 4a). In general, measures of genetic divergence between geographically adjacent strata were in agreement across all metrics used, and only chi square failed to support significant subdivision between the 2 geographically adjacent regions represented by CAL and WAL (see Supplementary Appendix 4 online). Pairwise measures of differentiation among a priori strata based on mtDNA sequences also indicated significant genetic differences for 7 out of 10 pairwise comparisons (Table 4a).

The WOMBSOFT analysis indicated the presence of significant genetic boundaries at Buldir Pass between WAL and CAL, and between EAL and GOA, but did not find genetic discontinuity between CAL and EAL (Figure 1b). In the western extent of the study area, putative genetic boundaries were also indicated within the RUS region separating the Kuril Islands (KUR) and Karaginsky Gulf (KAR) from Kamchatka Peninsula (Figure 1b).

Table 4 Pairwise measures of genetic differentiation based on both mtDNA and nDNA among resident killer whales for both (a) a priori and (b) a posteriori geographic strata

(a)	GOA	EAL	CAL	RUS	WAL
GOA	—	0.057	0.824*	0.965*	0.898*
EAL	0.040*	—	0.905*	1.000*	1.000*
CAL	0.063*	0.033*	—	0.124*	−0.004
RUS	0.094*	0.046*	0.033*	—	0.000
WAL	0.085*	0.039*	0.036*	0.009	—
(b)	**GOA**	**EAL-TRI**	**CAL**	**WAL-RUS**	
GOA	—	0.180*	0.783*	0.962*	
EAL-TRI	0.074*	—	0.915*	1.000*	
CAL	0.114*	0.031*	—	0.131*	
WAL-RUS	0.154*	0.036*	0.029*	—	

Estimates of F'_{ST} (nDNA) are presented below the diagonal and Φ_{ST} (mtDNA) are presented above the diagonal in (a) and (b) for the indicated population strata. Asterisks (*) indicate $P \leq 0.05$ based on 10 000 random permutations of the original data set. A complete list of all F_{ST} analogs based on nDNA presented in Supplementary Appendices 4 and 7 online.

STRUCTURE indicated the most likely number of subpopulations to be 5 when comparing the values of k (number of clusters) estimated by the methods of Pritchard et al. (2000) and Evanno et al. (2005; see Supplementary Appendix 5 online). As expected, running the model without prior information on sampling location suggested fewer genetic clusters ($k = 3$) with a lower average probability of assignment to the most likely cluster (without LOCPRIOR: mean ± SD = 0.577 ± 0.123; with LOCPRIOR: mean ± SD = 0.677 ± 0.143) reflecting the positive effect of the location prior on the model's ability to detect weak genetic structure. All STRUCTURE results (both with and without LOCPRIOR) identified the southern resident killer whale samples as a unique genetic cluster providing evidence of the model's ability to accurately identify discontinuous populations (inset, Figure 1a).

The distribution of genetic clusters based on the results of the STRUCTURE model incorporating the LOCPRIOR supported a population break within the Aleutian Islands between the a priori strata CAL and EAL at Samalga Pass (170°W), as well as a break between EAL and GOA west of Kodiak Island (Figure 1a). Whales sampled around the Trinity Islands (TRI) were assigned to 3 different genetic clusters. Within CAL, STRUCTURE assigned samples either to a cluster comprised of whales sampled in RUS-WAL ($n = 46$) or to a unique CAL cluster ($n = 48$) with nearly equally probability. No subdivision was indicated in the western regions of the study area within RUS or WAL (Figure 1a).

To evaluate the additional subdivisions suggested by WOMBSOFT and STRUCTURE, we revised boundaries and recalculated measures of genetic differentiation. RUS was divided into 3 regions (Kuril Islands (KUR), Kamchatka Peninsula (KAM), and Karaginsky Gulf (KAR)) and the Trinity Islands (TRI) separated from the other GOA samples (Figure 1a). Although WOMBSOFT suggested population subdivisions within the Russian samples, pairwise measures of genetic differentiation failed to support significant divergence between the discontiguous regions of KAR and KUR ($F_{ST} = 0.029$, $F'_{ST} = 0.054$, $P = 0.120$; see Supplementary Appendix 7 online). However, both of these regions were significantly differentiated from the adjacent WAL-KAM (KAR vs. WAL-KAM, $F_{ST} = 0.027$, $P = 0.007$; KUR vs. WAL-KAM, $F_{ST} = 0.040$, $P = 0.006$; see Supplementary Appendix 7 online), suggesting subdivision within the westernmost sampled regions. Significant divergence between the whales sampled around the Trinity Islands (TRI) and those in northern GOA ($F_{ST} = 0.029$, $F'_{ST} = 0.055$, $P = 0.009$), but a lack of differentiation between EAL and TRI ($F_{ST} = 0.008$, $F'_{ST} = 0.016$, $P = 0.115$) suggested that the genetic boundary for EAL may extend further east than that reflected by the a priori strata.

From these a posteriori analyses, we consider that the data support differentiation among 4 resident killer whale subpopulations (WAL-RUS, CAL, EAL-TRI, and GOA; Figure 1b). Measures of genetic differentiation among these a posteriori subpopulations supported the genetic divergence among these subpopulations based on both nuclear genotypic data ($F_{ST} = 0.031$, $F'_{ST} = 0.058$, $P < 0.001$) and mtDNA control region sequences ($F_{ST} = 0.904$, $P < 0.0001$; $\Phi_{ST} = 0.916$, $P < 0.0001$). Pairwise measures of genetic differentiation based on mtDNA sequence data did not support significant divergence among the a posteriori subdivisions west of Samalga Pass (Table 4b). This is likely attributable to the extremely low genetic diversity within the mtDNA control region resulting in fixed haplotypes that are shared among populations of piscivorous killer whales.

Genetic Structure of Transient Killer Whales

Pairwise measures of genetic differentiation among the 9 a priori strata of transient killer whales shared no significant mtDNA divergence (Φ_{ST}) among all strata east of Adak Island, except for PRI (Figure 2a; Table 5a). Transients sampled around the Pribilof Islands (PRI were also significantly differentiated from all strata east of Kamchatka Peninsula (Table 4a). There was no significant mtDNA differentiation between both TAN-RAT and TAN-KAM (Table 5a).

Table 5 Pairwise measures of genetic differentiation based on both mtDNA and nDNA among transient killer whales for both (a) a priori and (b) a posteriori geographic strata

(a)

GOA	KOD	EAL	UI	PRI	TAN	RAT	KAM	OKH
—	−0.010	−0.053	−0.045	0.574*	0.280*	0.257*	0.316*	0.574*
0.064	—	−0.027	0.040	0.594*	0.399*	0.346*	0.311*	0.661*
0.052*	0.061*	—	0.013	0.502*	0.272*	0.250*	0.341*	0.514*
0.034	0.080*	0.041*	—	0.624*	0.487*	0.436*	0.430*	0.698*
0.053*	0.033	0.019*	0.059*	—	0.632*	0.624*	0.179*	−0.020
0.034	0.125*	0.021	0.016	0.066*	—	−0.056	0.248	0.503*
0.009	0.086*	0.041*	0.076*	0.034*	0.067*	—	0.270*	0.518*
−0.007	−0.015	0.023	0.044*	0.013	0.041	−0.007	—	−0.015
0.060	0.129*	0.032	0.108*	0.098*	0.072*	0.022	0.049	—

(b)

KOD-GOA	EAL-TAN	UI	PRIB	OKH-KAM-RAT
—	−0.007	−0.033	0.605*	0.222*
0.041*	—	0.036	0.484*	0.158*
0.032	0.034	—	0.624*	0.259*
0.029*	0.024*	0.059*	—	0.212*
0.011	0.031*	0.065*	0.035*	—

Estimates of F'_{ST} (nDNA) are presented below the diagonal and Φ_{ST} (mtDNA) are presented above the diagonal in (a) and (b) for the indicated population strata. Asterisks (*) indicate $P \leq 0.05$ based on 1 000 random permutations of the original data set. A complete list of all F_{ST} analogs based on nDNA presented in Supplementary Appendices 4 and 7 online.

Estimates of differentiation based on nuclear microsatellite data revealed little or no significant genetic differentiation among some geographically adjacent a priori strata, suggesting larger subpopulations than the original strata tested (Table 5a; see Supplementary Appendix 4 online). Lack of significant differentiation among whales sampled west of Amchitka Pass (OKH, KAM, and RAT) provided strong evidence for a point of geographic subdivision at Amchitka Pass (179°E). Results also indicated a lack of genetic differentiation east of Kodiak Island (KOD and GOA). In the eastern Aleutians, significant nDNA differentiation was indicated between EAL and neighboring PRI to the north, but there was a lack of statistical support for the a priori split between EAL and TAN, to the west (Table 5a). Interestingly, significant genetic differentiation was apparent when comparing whales observed in spring assemblages around Unimak Island (UI) to the seasonally sympatric whales sampled in the EAL stratum (Table 5a). In general, all measures of genetic divergence between geographically adjacent strata concurred, with the exception of chi square which was marginally nonsignificant, failing to support the putative subdivision between RAT and TAN (see Supplementary Appendix 4 online).

WOMBSOFT analysis supported the broad patterns indicated above, highlighting both Amchitka Pass (179°E) as a significant genetic boundary between the western Aleutians (RAT) and the central Aleutians (TAN), and a zone of genetic differentiation within the Pribilof Islands (inset, Figure 2b). In Russian waters, the WOMBSOFT analyses suggested a latitudinal division across Kamchatka Peninsula (KAM) in the region of Avacha Bay (53°N) (inset, Figure 2b).

STRUCTURE without prior information on sampling location provided little evidence of genetic structure with all individuals being assigned to one of two clusters with nearly equal probability (mean ± SD = 0.546 ± 0.028). However, when location information (LOCPRIOR) was included, log-likelihood values suggest that transients in the sampled area most likely represent 3 genetic clusters (see Supplementary Appendix 6 online). While the evidence of genetic structure was weak, probabilistic cluster assignments for individual whales differentiated a small number of GOA samples ($n = 9$) and a subset of EAL samples ($n = 10$) around Unimak Island from all others (Figure 2a). Eight out of 10 of the individual whales assigned to the cluster around Unimak Island originated from the UI a priori stratum.

Lack of genetic differentiation among some a priori strata, as well as results from both WOMBSOFT and STRUCTURE, generally indicated fewer, larger population subdivisions than the 9 originally postulated (Table 5a). To reflect these results, regional population strata were redrawn into 5 larger a posteriori strata as follows: all samples west of Amchitka Pass (179°) were grouped together (OKH-KAM-RAT), samples from the central Aleutians (TAN) were grouped with those from the eastern Aleutians (EAL), and all samples from the Gulf of Alaska (KOD-GOA) were grouped into a single stratum (Figure 2b). Substructuring within the samples collected along the Kamchatka peninsula (KAM) was examined by comparing whales sampled within Avacha Gulf (AVA) to all others in KAM to further examine the zone of genetic discontinuity indicated by WOMBSOFT analyses.

Revised estimates of genetic differentiation (OKH-KAM-RAT, EAL-TAN, PRI, UI, and KOD-GOA; Figure 2b) supported the 5 a posteriori strata for both nuclear genotypes ($F_{ST} = 0.012$, $F'_{ST} = 0.034$, $P = 0.0009$; Table 5b) and mtDNA control region sequences ($F_{ST} = 0.271$, $P < 0.0001$;

Φ_{ST} = 0.295, $P < 0.0001$; Table 5b). Genetic differentiation among the Russian regions, including Avacha Gulf (AVA), were not significant (F_{ST} = 0.012, P = 0.183), most likely reflecting a lack of power due to extremely small sample sizes in this region for transient whales at the current time (AVA, n = 4).

Discussion

Using a suite of 26 microsatellite loci and a large number of georeferenced samples, we have provided the most comprehensive study of killer whale population genetic structure in the North Pacific to date. Analysis of molecular genetic data revealed significant levels of population genetic subdivision within the 2 predominant ecotypes of the genus *Orcinus* across the northern North Pacific using both mitochondrial control region sequences and nuclear microsatellite genotypes. Strong evidence of genetic divergence among neighboring geographic regions indicated multiple populations within the currently recognized stocks for both resident and transient killer whales. However, patterns of population genetic subdivision suggested some notable differences in the geographic structuring of populations between the 2 ecotypes.

Genetic Divergence among Ecotypes

Estimates of genetic distance between the 2 predominant North Pacific ecotypes indicate negligible levels of gene flow between ecotypes, confirming the findings of previous studies of ecotypic variation, and highlighting the genetic and demographic isolation of these 2 divergent evolutionary lineages in the North Pacific (Hoelzel and Dover 1991; Hoelzel et al. 2007; Morin et al. 2010; Pilot et al. 2010). This study more than doubled the total number of killer whale samples representing Alaska and Russia compared with previous studies (Hoelzel et al. 2007; Pilot et al. 2010) and substantially increased the number of polymorphic microsatellites from 16 to 26 loci. Recently, analysis of mitogenome sequences demonstrate phylogenetic sorting of ecotypes and suggest that transient killer whales should be elevated to full species status (Morin et al. 2010). The lack of shared mtDNA haplotypes and the significant genetic differentiation of nDNA data in this study support these findings and highlight the contemporary genetic divergence of the 2 ecotypes.

Geographic Structure of North Pacific Resident Killer Whales

Our analyses of the resident killer whale data set supported the existence of 4 longitudinally divided subpopulations across the North Pacific and Bering Sea. The eastern Aleutians subpopulation appears to diverge from the northern Gulf of Alaska in the waters around Kodiak Island. The 2 other major points of population subdivision coincide with 2 major island passes: Samalga Pass and Buldir Pass. The presence of population subdivision at Samalga Pass indicated by Bayesian cluster analysis of nDNA genotypic data was supported by a striking shift in the frequency of mtDNA haplotypes and also supported by all pairwise measures of genetic differentiation examined for resident killer whales. Samalga Pass has previously been recognized as a physical and biogeographic boundary between the eastern and central Aleutians (Ladd et al. 2005). WOMBSOFT analyses also indicated the presence of 2 possible genetic boundaries within Russia. Pairwise measures of genetic divergence supported genetic discontinuity between Kamchatka Peninsula and the Kuril Islands; however, there was a lack of evidence of genetic differentiation between the 2 noncontiguous regions separated by KAM (KAR and KUR), which may be attributable to small sample sizes (7 and 6, respectively). These major geographic subdivisions within the resident killer whale ecotype are consistent both with direct evidence of individual movements and with the geographic extent of social networks (Fearnbach H et al., unpublished data) and are supported by broad regional differences in both stable isotopes and persistent organic pollutants suggesting that differences in prey across the northern North Pacific may be a driving factor shaping population subdivisions (Krahn et al. 2007).

According to nDNA data, the point of subdivision between resident killer whales in the northern Gulf of Alaska (GOA) and the eastern Aleutians is in the region of Kodiak Island. Despite the indication of a genetic boundary west of the Trinity Islands, pairwise comparisons among strata suggest that whales sampled in this region (TRI) were significantly differentiated from GOA and most likely continuous with the eastern Aleutians subpopulation. Direct observations of photographically documented killer whales indicate a single population in the northern GOA spanning the waters from southeastern Alaska to Kodiak Island (Matkin 1997; Matkin et al. 1999), which is socially and spatially distinct from whales further west (Fearnbach 2012; Matkin et al. 2007). Association data and acoustic analyses also support an eastern Aleutian population of resident killer whales that interacts infrequently with Gulf of Alaska animals (Fearnbach 2012; Matkin et al. 2007). However, recently acquired data from satellite transmitter tags highlight marked seasonal differences in the movement patterns of whales in the northern and eastern Gulf of Alaska, as well as differences in core areas among matrilines (Matkin CO et al., unpublished data). These data emphasize the extreme mobility of these animals and underscore the limitations of inferring fixed boundaries from instantaneous samples.

Geographic Structure of North Pacific Transient Killer Whales

In contrast to the longitudinally defined geographic subpopulations of the resident killer whales, population genetic boundaries for transient killer whales indicate a few large geographic subdivisions, interspersed with smaller neighboring or seasonally sympatric subpopulations. As with the resident killer whales, genotypic data indicate that the waters around Kodiak Island likely represent the easternmost point of subdivision between EAL and GOA. Direct data on the movements of transient killer whales also support population differences between the eastern Aleutians and the Gulf

of Alaska (Matkin et al. 2007; Durban et al. 2010; Matkin et al. 2012). The westernmost subpopulation extends further east than that resolved from the resident genotypic data, encompassing both Russian areas (OKH and KAM) and those of the Rat Islands in the Aleutians, extending as far east as Amchitka Pass (179°W; Figure 2b). Pairwise measures of genetic differentiation indicated significant divergence between the neighboring a priori strata of Tanaga and Rat Island groups, supporting this as a significant place of genetic subdivision between the central and western Aleutians. It is important to note, however, that limited sample sizes in the western reaches of the study area restrict the resolution of population genetic structure west of Amchitka Pass and additional samples would greatly enhance our ability to determine contemporary levels of gene flow among the western Aleutians and Commander Islands.

Within the eastern/central Aleutians, our analyses provided strong evidence for multiple populations with a seasonal co-occurrence. Nuclear microsatellite data suggest the presence of 1 larger population cluster extending from the western GOA to Amchitka Pass, as well as a smaller sympatric subpopulation around Unimak Island. Observations of transients around Unimak Island in spring have revealed aggregations of killer whales that are distinct in acoustic call repertoire, patterns of association, and timing of occurrence compared with those further west (Matkin et al. 2007; Durban et al. 2010; Barrett-Lennard et al. 2011). During May and early June, concentrations of transient killer whales have been observed intercepting and preying on northward-migrating gray whales in the waters around Unimak Island (Barrett-Lennard et al. 2011). Genotypic data in this study were found to support the a priori hypothesis that whales observed in these spring foraging assemblages around Unimak Island are significantly divergent from some conspecifics sampled in the summer months in the adjacent eastern Aleutians. These signals of fine-scale sympatric genetic clusters may reflect social or ecological specializations occurring on a relatively small scale, or temporary/seasonal sympatry of killer whale populations during the summer months.

Unlike the fish-eating ecotype, the EAL subpopulation of transient killer whales was found to be genetically distinct from those around the adjacent Pribilof Islands in the Bering Sea based on both nDNA and mtDNA. A small number of photographically documented movements between EAL/UI and PRI as well as ongoing social network analyses support the existence of 2 neighboring strata that are connected by infrequent movements of individual whales (Wade P and Durban J, unpublished data). Killer whales are physically capable of undertaking extensive movements (Durban and Pitman 2011), likely responding to changes in prey availability, social requirements, or physiological constraints. Although satellite telemetry data and direct observations have demonstrated the capability of long-range movements by transient killer whales (Goley and Straley 1994; Matkin et al. 2012), individual resightings in our study region suggest seasonally based site fidelity with an average maximum straight line distance of only 95 km (minimum 2 km and maximum 507 km; Durban J, unpublished data) between repeated sightings across consecutive years. This indicates that although individual whales may not remain year-round in a given area, they are predictable in returning to seasonal prey aggregations (Durban et al. 2010). Seasonal changes in the abundance and distribution of key prey species may affect the degree of geographical overlap of neighboring subpopulations resulting from short-term convergence on prey aggregations.

Factors Shaping the Structuring of Killer Whale Populations

Marked seasonal variability in prey availability has been linked to temporal movements of transient killer whales in the North Pacific, often coinciding with seasonal concentrations of prey (Baird and Dill 1995; Matkin et al. 2002; Matkin et al. 2007; Dahlheim et al. 2009; Barrett-Lennard et al. 2011). For example, peak abundance in transient killer whale sightings at the Chiswell Island Steller sea lion rookery (Kenai Fjords) coincided with the peak in pinniped abundance (Maniscalco et al. 2007), and killer whale sightings around Unimak Island declined rapidly at the end of May following the migration of the majority of gray whale females and young-of-the-year calves (Barrett-Lennard et al. 2011). Stable isotope analyses further support observational data suggesting seasonal changes in the primary prey consumed by transient killer whales (Krahn et al. 2007). Partial sympatry in killer whale populations has also been described in the North Atlantic where population structuring appears to be largely driven by prey specialization (Foote et al. 2009, 2011). Among piscivorous killer whales in the eastern North Atlantic, potential geographic contact zones have been identified based on data from seasonal prey movements (Foote et al. 2011). Such temporal and spatial convergence of mobile predators not only provides incidental opportunities for male-mediated gene flow but also provides unique opportunities for ecological specialization.

Both killer whale ecotypes exhibited a lack of genetic differentiation between the northern and southern sides of the Aleutian Islands on the continental shelf. Despite the defining ecological differences inherent to the 2 killer whale ecotypes, both represent apex predators within the marine ecosystem, and factors such as prey preferences and distribution of preferred prey are likely responsible for shaping geographical population subdivisions. Regional dietary differences characterized for populations of other North Pacific marine mammals reflect similar geographic patterns to the genetic seascape described here for killer whales. Both humpback whales (*Megaptera novaeangliae*) and Steller sea lions foraging at a similar trophic level to resident killer whales exhibit regional differences in diet across the northern North Pacific that are largely correlated with longitude (Sinclair and Zeppelin 2002; Sinclair et al. 2005; Witteveen et al. 2009). A study of humpback whales using stable isotope ratios to infer regional differences among summer feeding grounds indicated a significant break in the western GOA representing a longitudinal shift in prey preferences from fish in the northern GOA to zooplankton in western GOA (Witteveen et al. 2009). That study also revealed dietary differences between

the eastern Aleutians and regions to the west (including the central and western Aleutians and the Commander Islands). Similarly, resident killer whales in Alaska also exhibited an east-to-west gradient in carbon and nitrogen isotope ratios between the GOA and the central Aleutian Islands suggesting regional prey differences (Krahn et al. 2007). Steller sea lions also exhibit marked regional differences in both population trends and prey preferences. Studies of Steller sea lion dietary differences among Aleutian Island rookeries found that diets east of Samalga Pass were more diverse and dominated by walleye pollock (*Theragra chalcogramma*) and salmon (*Oncorhynchus* spp.), compared with diets west of Samalga Pass that were heavily dominated by Atka mackerel (*Pleurogrammus monopterygius*) (Sinclair and Zeppelin 2002).

This longitudinal point of division also separates regions experiencing contrasting population trends within the endangered western stock of Steller sea lions (York et al. 1996; Sinclair and Zeppelin 2002; Call and Loughlin 2005). The identified geographic zone of differentiation among regions located at Samalga Pass corresponds with the geographic break described in this study for resident killer whales (supported by both mtDNA and nDNA data), which are likely feeding at the same trophic level as Steller sea lions and on some of the same prey. Resident killer whales have been observed feeding on salmon in the eastern Aleutians and on Atka mackerel in the central Aleutians (Wade P, Durban J, unpublished data). Both seabird (Jahncke et al. 2005) and zooplankton (Coyle 2005) species distributions also divide at Samalga Pass and it is thought that this area forms a key physical and biogeographic transition zone between the more coastal (or shelf-dominated) ecosystems of the eastern Aleutians and the more oceanic ecosystems of the central Aleutians (Ladd et al. 2005).

Interestingly, the observed patterns of geographic structuring described in this study for transient killer whales failed to support a significant subdivision between the eastern and central Aleutians around Samalga Pass. As apex predators, these killer whales are one step further removed from the direct effects of bottom-up structuring, described above. Although the tertiary consumers on which they prey may exhibit regional differences in population demographics and prey specializations, it is plausible that such effects become increasingly diluted at the top of the food chain, and other factors such as seasonal prey preferences and culturally transmitted prey specializations may assume significant roles in population structuring.

Management Implications

The patterns of genetic structure presented in this study provide strong evidence for the existence of multiple subpopulations of killer whales across the northern North Pacific, highlighting the need to revisit current stock designations. Killer whales in the northern North Pacific are impacted through both direct and indirect interactions with commercial fisheries. Evidence of population differentiation in this highly mobile species is a critical component for evaluating the impacts of incidental bycatch and estimating predator–prey relationships. A revision of the stock structure could have management implications for fisheries bycatch of resident killer whales in Alaska. Similarly, the geographic subdivision of transient killer whale populations may have implications for interpreting the role of killer whale predation in the decline and lack of recovery of Steller sea lions. However, these data also emphasize the need for additional individual-based data to inform fine-scale genetic analyses in areas such as Unimak Island and the Gulf of Alaska where multiple genetic clusters were indicated. Future individual-based analyses integrating direct observations and genetic data are necessary to resolve the temporal and spatial aspects of genetic structuring, and further our understanding of the localized role of killer whales as top predators and competitors in North Pacific ecosystems.

Supplementary Material

Supplementary material can be found at http://www.jhered.oxfordjournals.org/.

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Appendix 2
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