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 24120114-N
Final Report

John W. Durban and Craig O. Matkin

North Gulf Oceanic Society
3430 Main St. Suite B1
Homer, Alaska 99603

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Study History: In 1984, The North Gulf Oceanic Society initiated a monitoring program for killer whales (*Orcinus orca*) in Prince William Sound, which has continued with various funding support for 40 years. For the years 1984 to 1988, the work was partially funded by a variety of non-profit foundations and government grants. Following the *Exxon Valdez* oil spill in 1989, killer whales were monitored in Prince William Sound with funding from the *Exxon Valdez* Oil Spill Trustee Council in 1989, 1990, and 1991 and in 1993 (Dahlheim and Matkin 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. (1994). This monitoring project continued with support from the *Exxon Valdez* Oil Spill Trustee Council until 2021, as detailed in the projects listed below. Trustee Council funding for this project was significantly reduced for 2022-2023 and eliminated for the remainder of this reporting period. This current report adds to these previous studies to extend our monitoring time series from 1984 through 2024 (the latter two years supported by other funding sources). The final reports for the previous projects are available from the Alaska Resources Library and Information Services or from the North Gulf Oceanic Society as:

Dahlheim, M. E., and C. O. Matkin. 1993. Assessment of injuries to killer whales in Prince William Sound. Marine mammal study number 2. *Exxon Valdez* oil spill state/federal natural resource damage assessment final report (No. PB-96-194642/XAB). National Marine Fisheries Service, Seattle, Washington, USA. Alaska Fisheries Science Center.

Dahlheim, M. E. 1994. Assessment of injuries and recovery monitoring of Prince William Sound killer whales using photo-identification techniques. Restoration project 93042/94092. *Exxon Valdez* oil spill restoration project final report. No. PB-96-194667/XAB. National Marine Fisheries Service, Seattle, Washington, USA. Alaska Fisheries Science Center, 1994.

Matkin, C.O. 1994. An observer's guide to the killer whales of Prince William Sound. Prince William Sound Books, Valdez, Alaska, USA.

Matkin, C. O., G. Ellis, L. Barrett Lennard, H. Yurk, E. Saulitis, D. Scheel, P. Olesiuk, and G. Ylitalo. 2003. Comprehensive killer whale investigation. *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project 00112), North Gulf Oceanic Society, Homer, Alaska.

Matkin, C. O., G. Ellis, E. Saulitis, D. Herman, R. Andrews, A. Gaylord, and H. Yurk. 2010. 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.

Matkin, C. O., G. Ellis, E. Saulitis, D. Herman, R. Andrews, and A. Gaylord. 2013. 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.

Matkin, C. O., D. W. Olsen, G. Ellis, G. Ylitalo, and R. Andrews 2018. Long-term killer whale monitoring in Prince William Sound/Kenai Fjords. Long-Term Monitoring Program (Gulf Watch Alaska) Final Report (*Exxon Valdez* Oil Spill Trustee Council Project 16120114-N). *Exxon Valdez* Oil Spill Trustee Council, Anchorage, Alaska.

Matkin, C. O., J. Durban, D. Olsen, H. Myers, and G. Ellis. 2023. Long-term killer whale monitoring in Prince William Sound/Kenai Fjords. Exxon Valdez Oil Spill Long-term Monitoring Program (Gulf Watch Alaska) Final Report (*Exxon Valdez* Oil Spill Trustee Council Project 21120114-N), *Exxon Valdez* Oil Spill Trustee Council, Anchorage, Alaska.

Abstract: We continued photo-identification studies to assess changes in killer whale populations following the 1989 *Exxon Valdez* oil spill. Both the AT1 transient pod and the AB resident pod suffered significant losses following the spill and neither has recovered. The AT1 population lost half of their 22 individuals immediately following the spill and continued to lose individuals since. During this reported period, the AT1 pod lost their youngest whale for a total of just six whales remaining, continuing this pod's extinction trajectory. In contrast, after declining from 26 to 16 whales following the oil spill, AB pod had been slowly recovering to a post-spill high of 22 whales in 2015. However, AB pod declined precipitously following the 2014 - 2016 marine heatwave in the Gulf of Alaska, losing eight individuals and erasing 30 years of post-spill recovery. New population dynamics modeling showed that the post-heatwave decline was driven by both acute reduction in survival and delayed reduction in body size and related fecundity, adding only four new individuals by 2024. This study demonstrates how environmental variability affected the post-oil spill recovery of these long-lived top predators and demonstrates the need for continued monitoring to understand recovery potential for spill-affected killer whales.

Key words: Acoustics, diet, Kenai Fjords, killer whale, northern Gulf of Alaska, *Orcinus orca*, photogrammetry, Prince William Sound, population dynamics, resident, transient

Project Data: All required datasets are updated on the Gulf of Alaska Data Portal (https://gulf-of-alaska.portal.aoos.org/#metadata/2f42dd1c-d67a-4c49-8c2e-1d63387e0ad0/project). The photographic and acoustic files are very large and could not be uploaded and accessed with a browser easily. They were supplied to Axiom Data Science via a hard drive. Data from this reporting period acoustic recordings, surveys/encounters, and field identification photos.

The data custodian is Carol Janzen, Director of Operations and Development, Alaska Ocean Observing System, 1007 W. 3rd Ave. #100, Anchorage, AK 99501, 907-644-6703. janzen@aoos.org.

Data are archived by Axiom Data Science, a Tetra Tech Company, 1016 W. 6th Ave., Anchorage, AK 99501.

Citation:

Durban, J. W., and C. Matkin. 2025. Long-term killer whale monitoring in Prince William Sound/Kenai Fjords. *Exxon Valdez* Oil Spill Long-term Monitoring Program (Gulf Watch Alaska) Final Report (*Exxon Valdez* Oil Spill Trustee Council Project 24120114-N), *Exxon Valdez* Oil Spill Trustee Council, Anchorage, Alaska.

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EXECUTIVE SUMMARY

Population monitoring of killer whales (*Orcinus orca*) in Prince William Sound and Kenai Fjords, Alaska, has occurred annually since 1984. This report covers the three-year period from 2022 to 2024. We focused on two groups of killer whales of strategic interest because they were directly injured by the *Exxon Valdez* oil spill: the AT1 population of transients and the AB pod of residents. To provide a comparative context for assessing recovery, we also monitored other pods within the southern Alaska resident population, of which AB pod is part. Major findings include: (1) the AT1 population continues to decline toward extinction, (2) AB pod suffered a major setback in their recovery from the spill with a population drop of 36% following the northeast Pacific marine heatwave (2014-2016), and (3) reduced growth of individuals during and following the heatwave indicates that ecosystem perturbations can have prolonged sub-lethal effects on health in addition to acute lethal impacts, particularly through reductions in female fecundity associated with smaller body sizes.

In the current study period, a total of 128 survey days were spent on the water in May-July across the three years. There were 88 encounters with resident killer whales and nine encounters with transients. The AT1 population numbered 22 animals prior to the spill but dropped to half of this abundance by the early 1990s. During this reported period their abundance dropped from seven to just six whales, with the loss of the youngest remaining whale. This population is headed toward extinction because the four remaining females are likely beyond reproductive ages.

The AB pod declined from 26 to 16 whales following the oil spill, but in contrast to the AT1 population was slowly recovering to a post-spill high of 22 whales in 2015. However, AB pod declined precipitously to its lowest recorded abundance of just 14 whales in 2017, and has since been slowly growing, increasing to 18 in 2024. AB pod is part of the larger AB acoustic clan of residents. The AB clan increased by 8% in combined abundance during the reporting period. In contrast, the abundance of index pods in the inshore-tending AD acoustic clan increased by only 3% over the reporting period, highlighting the varied population responses that are likely underpinned by ecological differences.

This latest decline of AB pod came at the end of a marine heatwave between 2014 and 2016 that has had acute and prolonged impacts on the Gulf of Alaska ecosystem and erased 30 years of post-spill recovery of AB pod. New population dynamics modeling showed that this was driven by both acute declines in survival and delayed declines in fecundity and was mirrored by some other resident pods, particularly other pods in the related AB acoustic clan. These data demonstrate that the impacts of environmental variation can permeate up through the marine ecosystem to these long-lived top predators and demonstrates the need for continued monitoring to understand how environmental variation will affect recovery potential.

Reduced growth of individuals during and after the heatwave was detected through analyzing photogrammetry data on individual size-at-age, indicating sub-lethal effects that will likely lead to prolonged impacts on population recovery. Specifically, we estimated a high probability of a positive relationship between asymptotic length of females and the probability of calving. Smaller body sizes confer reduced energy stores to support pregnancies and lactation. As such, we anticipate the impacts of the heatwave to be prolonged, particularly if females face the challenge of reproduction during future periods of nutritional limitation.

After 2022, the Trustee Council reduced funding for this project in 2023 and removed funding entirely for the remainder of the 2022-2026 Gulf Watch Alaska funding period. This reduction and then cessation in funding prevented us from addressing all the objectives proposed for this five-year funding period. Nonetheless, we obtained a limited amount of other support to complete three years of data collection and to conduct a reduced suite of data analysis tasks reported here.

INTRODUCTION

Population monitoring of killer whales (*Orcinus orca*) in Prince William Sound and Kenai Fjords, Alaska, has occurred annually since 1984. The existence of data prior to the *Exxon Valdez* oil spill in 1989 has made it possible to determine that groups of whales observed in the slicks of oil suffered significant subsequent mortalities. This includes both whales of the fish-eating resident ecotype, specifically AB pod, and whales of the mammal-eating transient ecotype, specifically the AT1 population (Matkin et al. 2008). The AT1 population is not recovering and is headed toward extinction (Matkin et al. 2012). The AB pod was recovering slowly until a decline during the last reporting period (Matkin et al. 2023) and has still not reached pre-spill numbers. While our priority is monitoring population changes over time, we are also investigating the causes of these changes.

This latest decline of AB pod came at the end of a marine heatwave during 2014-2016 that has had acute and prolonged impacts on the Gulf of Alaska ecosystem (Suryan et all. 2021). This highlighted the need to further understand how environmental variation will affect their recovery potential. As top predators, killer whale populations reflect the integrated status of the trophic levels that support them (Ford et al. 2010). During the northeast Pacific marine heatwave (PMH) the Gulf of Alaska experienced some of the most severe impacts on marine species across trophic levels (Suryan et al. 2021), including declines in commercial catches of Chinook salmon (*Oncorhynchus tshawytscha*), a known prey species for the resident ecotype of killer whales in this region (Saulitis et al. 2000). Based on data presented in the last reporting period (Matkin et al. 2023), we recently published a paper adding more detail on the diet of resident killer whales in our study area (Van Cise et al. 2024) with another paper is currently in review (see Appendix 1). Together, these papers describe a varied diet of primarily Chinook, chum (*Oncorhynchus keta*), and coho (*Oncorhynchus kisutch*) salmon, with dietary contributions also from non-salmonid fish including Pacific halibut (*Hippoglossus stenolepis*), arrowtooth flounder (*Atheresthes stomias*), and sablefish (*Anoplopoma fimbria*).

If prey resources were impacted by the PMH, then impacts to killer whale population dynamics and individuals' nutritional health may expected, and we therefore conducted investigations into both. Based on updated photo-identifications through 2024 we undertook a new analysis of population dynamics of AB pod and 9 other regularly sighted "index" pods of resident killer whales to provide a comparative context for population dynamics in this changing ecosystem. A journal paper detailing the population dynamics of resident killer whales in the northern Gulf of Alaska, including the impacts of the marine heatwave is in preparation and progress is summarized here.

Recovery of depleted populations depends on successful reproduction (Moore 2023) to ultimately recruit new reproductive females into the adult population. As such, our population dynamics modeling investigated if there were effects on fecundity due to constrained nutritional status during the heatwave. In killer whales, reproduction is limited to a single calf every five years on average, during female reproductive ages between early teens and early forties (Matkin et al. 2014). This slow reproduction translates into smaller population growth rates compared to many marine mammals, and as such any negative impacts on reproductive success can significantly impact their recovery potential and lengthen already long recovery times. Smaller body sizes confer reduced energy stores to support pregnancies and lactation (Stewart et al. 2022), and there may be even longer-term impacts on fecundity if the growth of females was impacted as a result of nutritional stress during the PMH (e.g., Fearnbach et al. 2011, Groskreutz et al. 2019). We therefore also investigated changing patterns of growth and their effect on fecundity.

To investigate patterns of growth, we measured size of known-age whales using photogrammetry from drone-derived images (Durban et al. 2021), an approach we first implemented during the previous reporting period (Matkin et al. 2023). In this reporting period we continued to use this method towards the goal of quantifying health responses to ecosystem changes to further help diagnose the causes of population dynamics and predict future vulnerability. The full suite of photogrammetry analyses that we proposed have not yet been possible due to the shortened duration and reduced funding from the *Exxon Valdez* Oil Spill Trustee Council. However, with funding support from the U. S. Marine Mammal Commission we were able to advance a study investigating changes in whale growth and the impact of female size on their reproductive success.

OBJECTIVES

1) Photo-identification of all major resident pods, the AT1 transient group, and secondarily the Gulf of Alaska transients and Offshore killer whales. The spill-affected Prince William Sound/Kenai Fjords study area was our primary focus but contributed photographs from adjacent regions were used as possible to augment our work. All other objectives are based on this primary objective. The program included the extension of individual histories, updates of identification catalogues of individuals, and annual updates of population tables and development of long-term population dynamics analysis at appropriate intervals. (Primary Objective).

2) Collection of fish scale samples and marine mammal tissue from kill sites and collection of killer whale fecal samples coupled with genetic analysis to understand feeding ecology differences by location, by season, and between populations. (Core Objective).

3) Determine movements and distribution using strategically located remote SoundTrap hydrophone/recorders on submerged moorings. Annual analysis of calls provided detailed information on patterns of killer whales, specifically the seasonal timing of use by specific pods (Core Objective).

4) Use the innovative techniques of aerial photography from a drone platform to determine and compare morphometrics of individuals to assess individual body condition on an annual basis, monitor growth trends and to determine pregnancy rates and contrast those with recruitment rates derived from photo-identification (Core Objective).

5) Develop models that integrate changes in body size, body condition and population dynamics to provide more resolution on population status and identify mechanistic links with environmental and trophic covariates. Fit models to provide updates in each 5-year phase on whale population dynamics, compare population status with other killer whale populations and develop mechanistic explanations for changes to whale population status (Core Objective).

6) As an option if time allows, obtain genetic skin tissue samples when necessary to determine population/ecotype affiliations and to sample blubber at extended intervals to examine contaminant levels and compare with previous samples. (Secondary Objective).

Objectives 1-5 were partially addressed and objective 6 was not addressed due to the loss of funding after FY23.

METHODS

Data collection

Fieldwork during the 2022-24 study period was completed from the R/V *Natoa,* a 10.3 m inboard diesel-powered vessel, capable of 12 knots and sleeping up to four researchers. 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). We based field timing and search locations on current and historical sighting information to maximize the number of killer whale encounters. This information included passive acoustic detections (Myers et al. 2021) and satellite tag data (Olsen et al. 2018). Consequently, searches were centered in areas with historically high encounter rates with killer whales, unless sighting or detection information indicated changes in whale distribution. Whales were found visually, by listening for killer whale calls with a 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. An encounter was defined as the successful detection, approach and taking of identification photographs of killer whales.

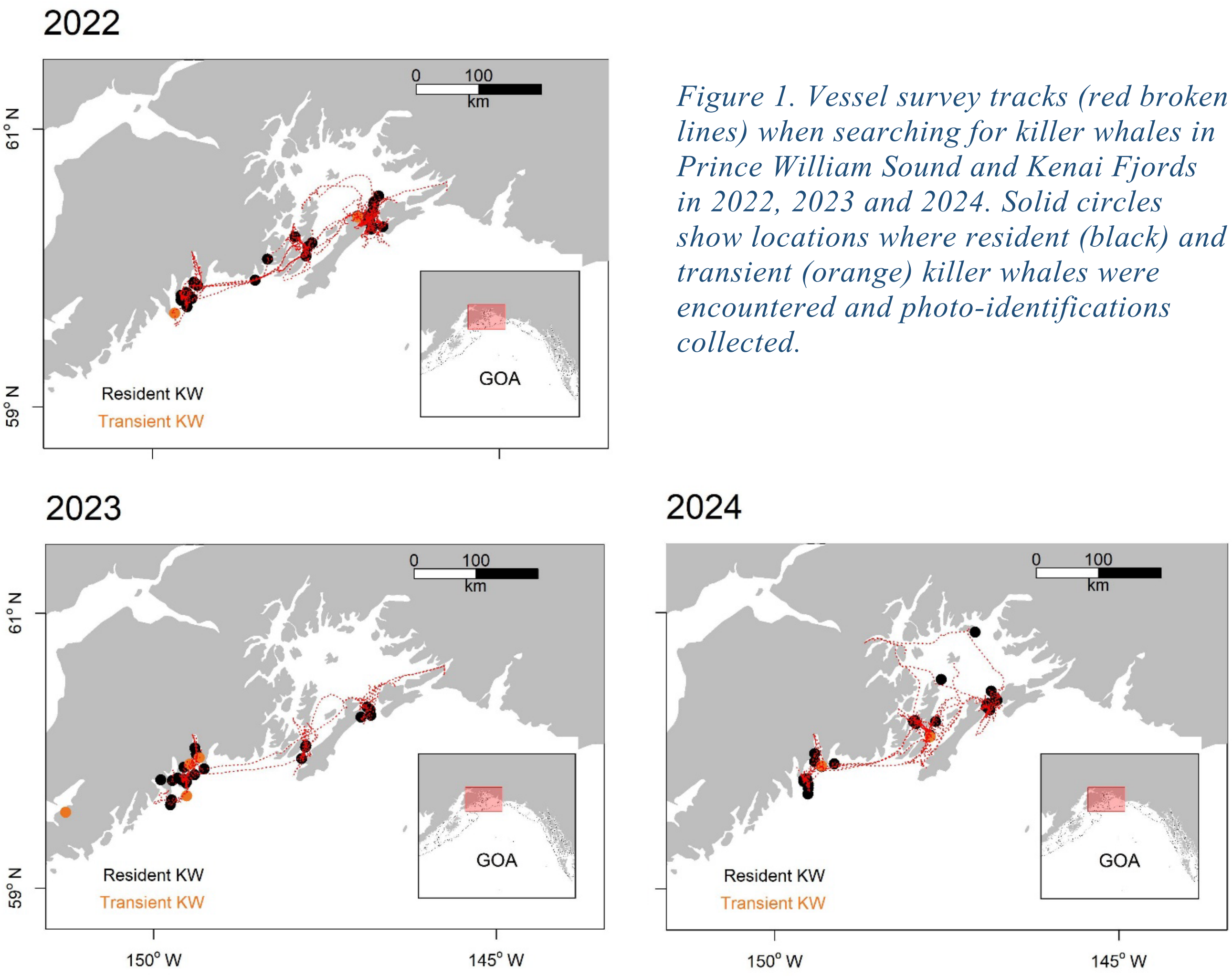

Figure 1. Vessel survey tracks (red broken lines) when searching for killer whales in Prince William Sound and Kenai Fjords in 2022, 2023 and 2024. Solid circles show locations where resident (black) and transient (orange) killer whales were encountered and photo-identifications collected.

To meet Objective 1, we continued to use standard killer whale photo-identification procedures detailed in Bigg et al. (1990) and Matkin et al. (1999). Photographs for individual identification were taken of the left side of each whale showing details of the dorsal fin and saddle patch. Digital images were taken at no less than 1/1000 second shutter speed using a Nikon D700 or D750 digital SLR camera and a 300mm f4 or 80-400 f4.5-5.6 autofocus lens. When whales were encountered, we systematically and slowly 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 ensure 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 using a Garmin GPS V that was downloaded each evening. The elapsed time and distance traveled were independently recorded daily. 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 periodically to reflect changes in data collection needs and emphasis. 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 composition and individual behaviors (i.e., feeding, resting, traveling, socializing, milling) were recorded by time and location. Directed observations of feeding behavior and collection and collection of killer whale prey and fecal material were made when possible.

To complete Objective 2, fish scales or flesh and killer whale fecal samples were collected using an extendable, fine-mesh dip net following visually detection of resident killer whale predation events or defecations, respectively. Sampling occurred opportunistically alongside each annual photo-identification census. Curtailed funding has prevented analysis of samples collected during the reporting period, but in the future, we hope to use these samples to generate data on both prey species identification using DNA analysis of fecal samples (Van Cise et al. 2024) as well as prey species identity and age from fish scale annuli (Saulitis et al. 2000). Sampling of prey was coupled with standard killer whale photo-identification procedures (Objective 1) to determine the identity of the population, the pod, and, in some cases, the individual whale, using existing photographic catalogues (Matkin et al. 1999).

Predation events by resident killer whales were identified by visual cues such as erratic movements of widely spaced individuals. As in our previous study (Saulitis et al. 2000), predation events were accompanied by noticeable whale surface activity that triggered our movement to the kill site where we attempted collection of scale samples. We also were successful in obtaining scale and fecal samples by following individuals (or female/calf pairs) for extended periods during foraging and waiting for successful feeding or defecation to occur.

To complete Objective 3, we placed Ocean Systems "SoundTrap" autonomous recorders in 32-45 m of water in five remote hydrophone locations: Entrance to Resurrection Bay, Lower Montague Strait, Hinchinbrook Entrance, Kachemak Bay and Marmot Island (Fig. 2). All three areas were known to be important for killer whales at least seasonally from earlier telemetry data (Olsen et al. 2018). The year-round recordings have previously enabled year-round patterns of distribution to be elucidated (Myers et al. 2021), which was not possible with shorter term telemetry.

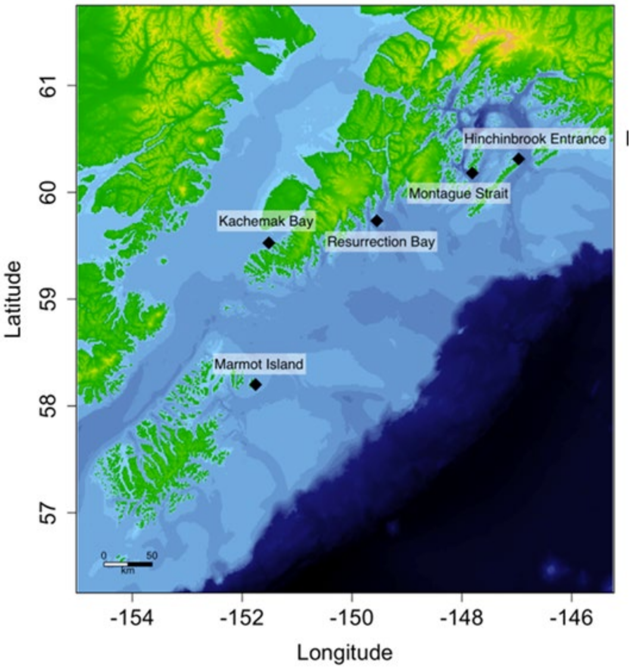

Figure 2. Location of five remote hydrophone stations.

To complete Objectives 4 and 5, we used drone photogrammetry (Durban et al. 2015, Groskreutz et al. 2019, Durban et al. 2021) as the field technique to collect images (Fig. 3). Following a successful pilot year in 2021 (Matkin et al. 2023), further images were collected in each of the 2022 and 2024 field seasons, including data collection both in the Kenai Fjords and in Prince William Sound. Vertical images were collected from directly above killer whales using a custom research octocopter (APO-36, Aerial Imaging Solutions) launched and retrieved by hand from the flying bridge of the R/V *Natoa*. This large octocopter had sufficient lift to carry a full-frame camera (Sony A7R with 36MP resolution) in a vertical gimbal. A precise laser altimeter (<0.1% error, Dawson et al. 2017) was mounted on the same gimbal to enable measurements in pixels to be scaled to real size (using methods described in Durban et al. 2022). Measurements were linked to whales of known identity and reproductive histories (Fig. 4) by matching to an established photo-identification catalog (http://www.whalesalaska.org; e.g., Durban et al. 2015, Fearnbach et al. 2011, 2018, Groskreutz et al. 2019).

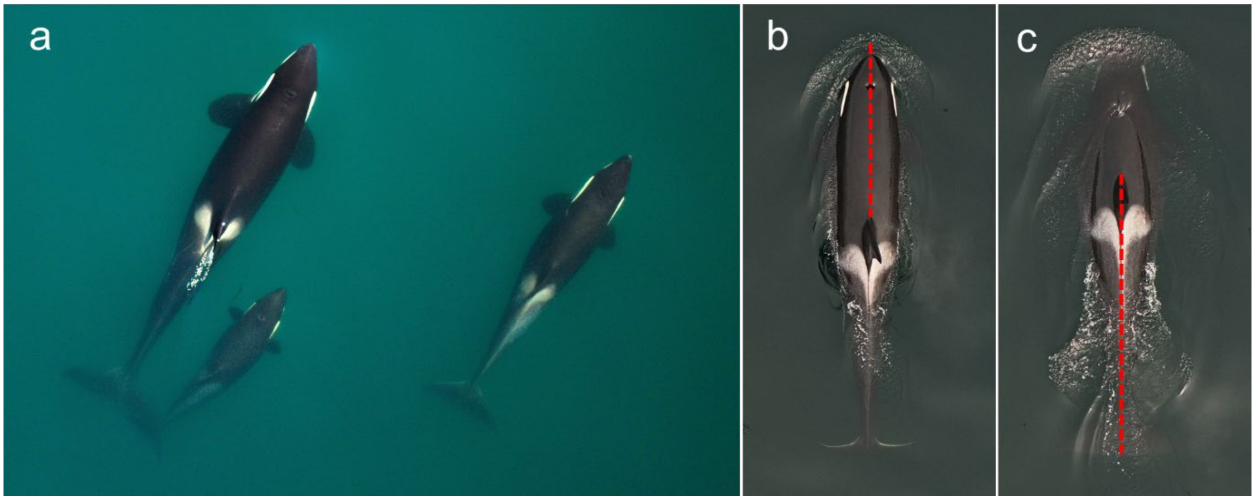

Figure 3. Vertical photographs taken directly above killer whales by an octocopter drone (average altitude 40 m, 130 ft). Left (a) shows an adult female with a calf and her older juvenile offspring. We derived length from the addition of two separate measurements: a snout to dorsal fin measurement (b) from the tip of the rostrum to the anterior insertion of the dorsal fin, and a dorsal fin to fluke measurement (c) from the anterior insertion of the dorsal fin to the central margin of the fluke notch. These measurements were typically from separate but often sequential images.

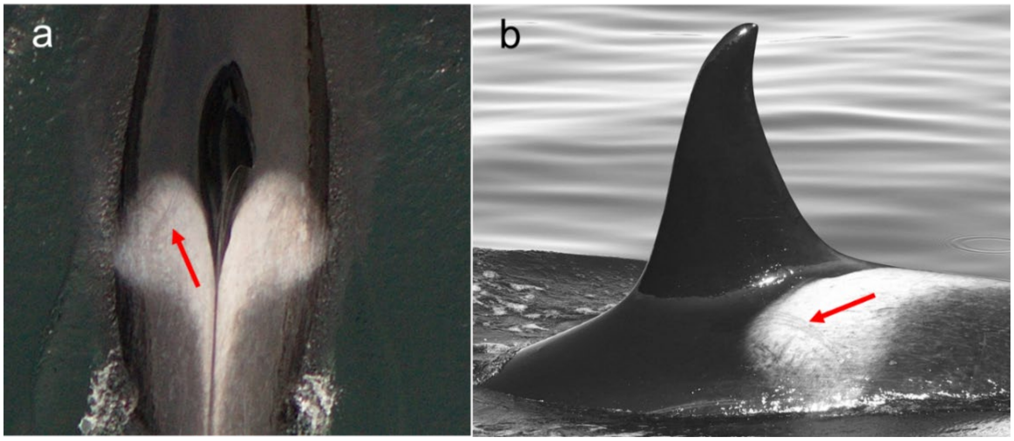

Figure 4. Unique scarring and pigmentation patterns on each whale's saddle patch was used to match aerial images (a, taken from altitude of 39m or 127ft) to our long-term catalog (http://www.whalesalaska.org) of boat-based photo-identification images (b) and therefore link length measurements to females of known age. Arrow in figures indicate the same distinctive scars on the saddle of AK9, an adult female born 1986 and the same whale shown in the measurement panel (Fig 2b, 2c).

Analytical methodology

To address Objective 1, digital images were examined using PhotoMechanic software (CameraBits Inc.) on a 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. The alphanumeric code used to label individuals 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). 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. All confirmed annual records were compiled into a master dataset of annual identifications. We have posted updated catalogues of individuals annually on our website (https://www.whalesalaska.org/).

For Objective 2, new analysis of prey samples or whale feces was not performed due to curtailed funding. For previous methods and results see Van Cise et al. (2024) and Appendix 1.

For Objective 3, new analysis of acoustic detections was not performed due to curtailed funding. For previous methods and results see Myers et al. (2021).

To address Objective 4. The freely available image processing program ImageJ (https://imagej.net/ij/) was used to generate whale length measurements in pixels from drone photographs. The steep surfacing angle of killer whales does not typically allow for a single accurate measurement of total length (TL) from a single image so we derived TL from two separate measurements: a snout to dorsal fin (SNDF) measurement from the tip of the rostrum to the anterior insertion of the dorsal fin, and a dorsal fin to fluke (DFFL) measurement from the anterior insertion of the dorsal fin to the central margin of the fluke notch (Fig. 3). These measurements were typically from separate but often sequential images when each respective body segment was flat and parallel to the water's surface. Estimates of TL were derived by adding the maximum SNDF and DFFL values of an individual whale collected within each short year-season sampling period, and this was assumed to represent the flattest description of an individual (Groskreutz et al. 2019, Kotik et al. 2023). Only length measurements of adult females and young whales have been generated to date due to curtailed funding. Measurements of body condition have not been completed due to curtailed funding.

To address Objective 5, we conducted new modeling of population dynamics to examine long term trends in survival and fecundity, in particular changes around the timing of the PMH. For this reason, we only included index pods that were documented regularly before and after the PMH (Matkin et al. 2023). Components for survival and fecundity were included in the same Bayesian model (following Ward et al. 2016), allowing both to contribute to our understanding of abundance trends. Temporal trends in survival were investigated by fitting a Cormack-Jolly-Seber mark-recapture model to our 41-year (1984-2024) time series of photo-identification data (Ward et al. 2016). Capture probabilities were modeled through fixed pod effects that allowed the average detectability to vary between pods, and time-varying random effects that allowed trends in the detectability separately for each pod in each year. Survival probabilities included fixed effects for the average annual survival rate of each age/sex class and a time-varying random effect that allowed variability in the survival of members of each pod separately in each year. Following Ward et al. (2009, 2016) six age/sex classes were defined: calves were less than one year of age, juveniles (1-9), young males (10-21), older males (22+), reproductive females (10-42), and post-reproductive females (43+). Additionally, whales of unknown sex, prior to having a calf or developing male secondary sexual characters, were classified as unknown subadults 10+ years of age.

In the same Bayesian model, we investigated temporal trends in fecundity using photo-identification data for females in index pods to provide records of births to females of known age. An age-structured model (Ward et al. 2016) was fit to annual records of females with and without new calves to describe the probability of a female calving in each year when she was available (i.e., alive, sexually mature, not in a known pregnancy year, and not resting in a year after calving). This model estimated the expected fecundity-at-age relationship (Matkin et al. 2014; Fig. 5), and time-varying random effects were incorporated to assess annual changes in calving probability of each pod, after accounting for different ages of females in each year.

The timing and strength of the annual effects for survival and fecundity were examined relative to the timing of the PMH, and we used a Bayesian approach to model fitting to estimate the probability that each annual effect departed from zero (indicating no annual departure). Uniform prior distributions were adopted for the main effect terms for pod-specific capture probability, age/sex class survival and coefficients describing a fourth-order polynomial function for the probability of calving over different ages (Ward et al. 2016, Fig. 5). The annual random effects terms for capture probability, survival and fecundity were each modeled using separate normal prior distributions on the logit scale, centered on zero (no annual effect) with a uniform prior on the random effect standard deviations for each pod to allow annual departures for each pod independently, if supported by the data. We used the package *nimble* (de Valpine et al. 2020) within the R environment for statistical computing (R version 4.4.2, The R Foundation for Statistical Computing) to fit the hierarchical model and estimate posterior distributions of model parameters using Markov chain Monte Carlo (MCMC) sampling. Inference was based on 100,000 MCMC samples of a single chain following a burn-in of 100,000, with the sample thinned to 10,000 values using every 10th MCMC value to reduce chain autocorrelation.

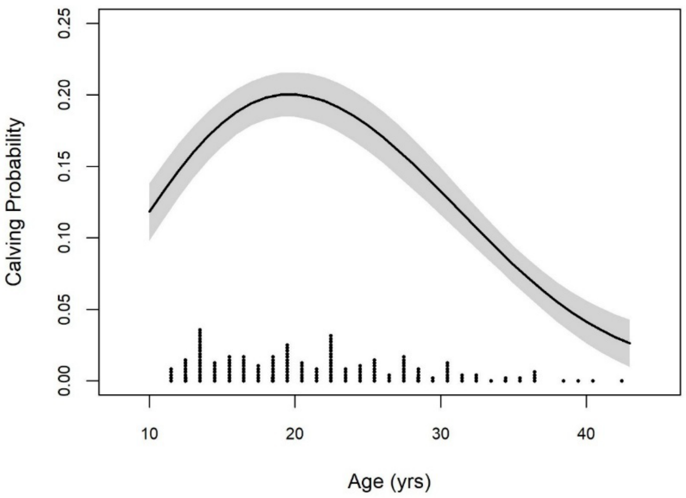

Figure 5. Bayesian posterior mean (line) and standard deviation (gray ribbon) of the expected calving probability of Alaska Resident killer whales against known age, along with the ages of 81 females at the time of 168 known births (dots). For females available to give birth (alive and not nursing a current calf), the probability of having a new calf was modeled as a fourth-order polynomial function of age (Ward et al. 2016).

We also analyzed photogrammetry data to investigate if there were additional sub-lethal effects of the heatwave due to declines in female length. We fit a growth curve model to the TL of females at ages known from long term photo-identification data. We included all whales known to be female through either direct observation of sexually diagnostic ventral pigmentation (Ford et al. 2011) or by repeated observations with a dependent calf. To estimate the shape of the curve at young ages (<10 yrs), we included younger whales of unknown gender, assuming no sexual dimorphism. Excluded from this dataset, however, were adult and sub-adult males, which can be distinguished from females after approximately 10 yrs of age based on "sprouting" of taller dorsal fins as males mature (Durban and Parsons 2006, Matkin et al. 2014). These sub-adult and adult males also diverge significantly from female body growth and attain longer body lengths (Fearnbach et al. 2011). We fit a Bayesian formulation of the flexible Richard's growth curve (Tjørve and Tjørve 2010, Fearnbach et al. 2011) to describe the asymptotic growth of females, with a random effect to incorporate individual departures from the average asymptotic length (e.g., Stewart et al. 2021). The model also allowed for repeated measures of the same whales in multiple season-year sampling periods to more precisely estimated their individual asymptotes. We fit the model using the package *nimble* (de Valpine et al. 2020) in R version 4.4.2. Estimates of the posterior distributions for model parameters, specifically the average growth curve and individual asymptotic lengths, were based on 100,000 MCMC samples of a single chain following a burn-in of 100,000, with the sample thinned to 10,000 values using every 10th value MCMC value. We then fit a generalized additive model (GAM) to the estimated mean of each individual's Bayesian posterior distribution for asymptotic length to explore the shape of the relationship with their age at the onset of the PMH in early 2014. The GAM was fit using the *mgcv* package (Wood 2017) in R version 4.4.2.

We then investigated if there was an effect of female size on fecundity. Individuals were allowed to depart from the typical fecundity schedule by introducing a random intercept term to the age-structured, time-varying model. This assumed the same pattern of fecundity-by-age for all individuals but allowed the level to be shifted up (more fecund individuals) or down (less fecund) from the average curve. This random intercept term was modeled as a linear function (on the logit scale) of each individual's asymptotic length, estimated from the growth curve model, although individual intercept terms were drawn from a normal distribution and were not constrained to fall directly on the regression line. We used a Bayesian approach to model fitting to estimate if the slope of the regression line differed from zero (indicating no relationship between female length and fecundity). This model was also fit using the package *nimble* (de Valpine et al. 2020) in R version 4.4.2. Estimates of the posterior distributions for model parameters, specifically the regression parameters and the probability of a non-zero slope, were based on 100,000 MCMC samples of a single chain following a burn-in of 100,000, with the sample thinned to 10,000 values using every 10th value MCMC value.

Analyses of body condition were not conducted due to curtailed funding. Comparisons of body condition and growth of Alaska Resident killer whales to other killer whale populations was not conducted due to curtailed funding. Models identifying mechanistic links between body condition, growth, population dynamics and environmental and trophic covariates was not conducted due to curtailed funding.

Objective 6 was not addressed due to curtailed funding.

RESULTS

Summary of effort and encounters

During the reporting period, 2022-2024, the R/V *Natoa* spent a total of 128 days on the water searching for killer whales along 10,498 km of track line for an average search distance of 82 km per day. Killer whales were encountered on 97 occasions, comprising 88 groups of residents and nine groups of transients (Table 1, Fig. 1).

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

Year	# Vessel survey days	# Encounters	Distance Surveyed (km)
2022	50	38	4320
2023	32	28	2234
2024	46	31	3944
TOTAL	**128**	**97**	**10498**

Population dynamics

Two groups of killer whales are of strategic interest because they are known to have been directly injured by the *Exxon Valdez* oil spill (Matkin et al. 2008): the AT1 population of transients and the AB pod of residents. Neither has recovered to their pre-spill numbers (Fig. 6). During this reporting period, the AT1 population declined from seven to just six whales, with the loss of the youngest remaining whale (AT3, a male born in 1984). This remains below their pre-spill high of 22 individuals. Further recruitment is not expected because the remaining females are beyond known reproductive age for killer whales. After declining from 26 to 16 whales following the oil spill, AB pod had been slowly recovering to a post-spill high of 22 whales in 2015. However, AB pod declined precipitously to its lowest recorded abundance of just 14 whales in 2017, and has since been slowly growing, increasing to 18 at the end of this reporting period in 2024. This latest decline of AB pod came at the end of a marine heatwave during 2014-2016 that has had acute and prolonged impacts on the Gulf of Alaska ecosystem and apparently erased 30 years of post-spill recovery of AB pod (Fig. 6).

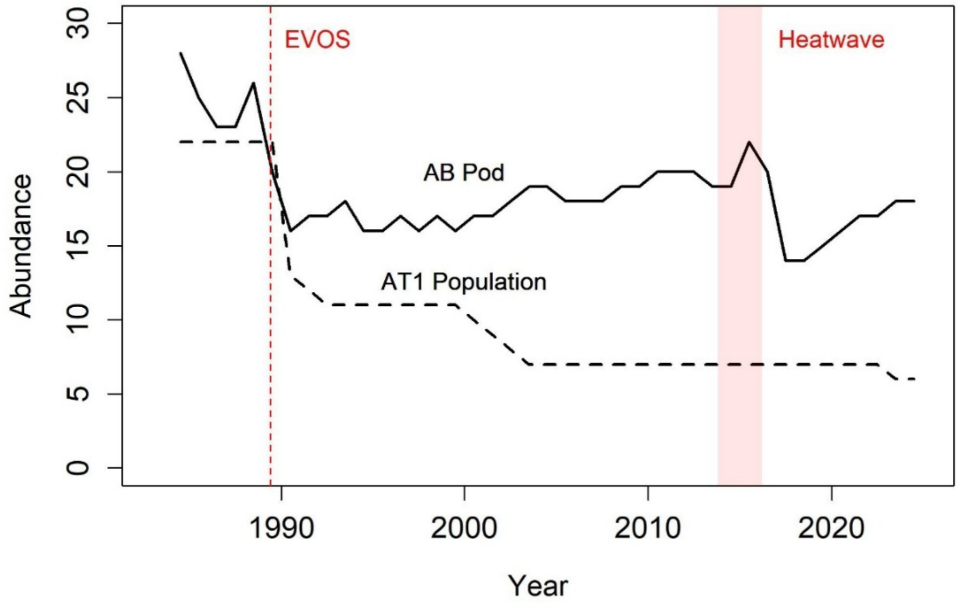

Figure 6. Number of killer whales (Abundance) in the AB pod and AT1 population from 1984 to 2024. The timing of the Exxon Valdez Oil Spill (EVOS) and the northeast Pacific marine heatwave are indicated in red.

To provide a comparative context for assessing recovery of AB pod during the 2022-2024 study period, we also monitored other pods within the southern Alaska resident population (totaling 200 whales in 2024). We have photographed more than 1000 individual whales in this population but we monitor the detailed dynamics of regularly seen "index pods" that were encountered and censused annually, or at least every other year. In the AB clan of acoustically related pods (Yurk et al. 2002), of which AB pod is part, all 5 index pods increased in abundance over the reporting period, increasing by 8% in combined abundance (Table 2). In contrast only

2/5 index pods in the AD clan increased in abundance and the others remained constant in size, representing a combined increase of just 3%.

Table 2. Recruitment, mortalities, and total number of killer whales in frequently seen "index" resident pods censused since the last report. Shading indicates pods from the AB acoustic clan (see Yurk et al. 2002), listed above those pods from the "AD" acoustic clan.

POD	Total 2021	# Calves since 2021	# Missing since 2021*	Year of last census	Most Recent Total
AB	17	3	2	2024	18
AB25	27	4	2	2024	29
AJ	44	6	2	2024	48
AJ08	19	1	0	2024	20
AI	10	1	0	2024	11
AB Clan sum	**117**	**15**	**6**		**126**
AE	18	3	3	2024	18
AK02**	20	0	0	2024	20
AK06	10	1	0	2024	11
AD08	10	0	0	2024	10
AD16	14	2	1	2024	15
AD Clan Sum	**72**	**6**	**4**		**74**

** Missing indicates likely death, but will be determined by future photo-identifications*
*** One matriline of the AK02 pod (AK10 matriline) not encountered in 2024, assumed constant*

Mortality and births, pre and post Pacific Marine Heatwave

We assessed survival for 292 different whales that were photo-identified in the index pods (Table 2) between 1984 and 2024, comprising 4214 annual photo-identification records. Average annual survival was very high across all age-sex classes, as would be expected for pods that have been experiencing growth for most of the past four decades (Matkin et al. 2014, Matkin et al. 2023). Not surprisingly, the survival rate was lowest for post-reproductive females and older males that are old by definition, and also for vulnerable young calves (Table 3).

Table 3. Average annual survival estimates for each age/sex class, presented as posterior median (95% probability intervals) from a Bayesian mark-recapture model. Seven age/sex classes were defined: calves were less than one year of age, juveniles (1-9), young males (10-21), older males (22+), reproductive females (10-42), post-reproductive females (43+) and unknown subadults 10+ years of age. Estimated annual effects allowed departures to these age-structured survival rates for each pod in each year (see Fig. 7). Survival rates (shaded) were lowest for calves, older males and post-reproductive females.

Age/ Sex Class	**Survival Rate** Posterior Mean	**Survival Rate** 95% Probability Interval
Calves	0.96	0.93-0.98
Juveniles	0.99	0.98-0.99
Unk Subadults	0.98	0.95-0.98
Young Males	0.99	0.98-0.99
Older Males	0.95	0.93-0.96
Reproductive Females	0.99	0.98-0.99
Post-reproductive Females	0.92	0.87-0.96

After the heatwave, most (7 of 10) index pods had strong evidence ($p > 0.75$, or three times the weight compared to no effect) of a decline below their average annual survival (after accounting for the expected age effects); five declined from 2016 to 2017 and three declined between 2015 and 2016 (Fig. 7). These pods comprised four of five AB clan pods and three of five AD clan pods. The largest AJ pod showed reduced survival in both 2015-2016 and 2016-2017, likely resolved due to the elevated statistical power provided by its larger numbers. The strongest evidence ($p = 1$) was for AB pod declining in survival between 2016 and 2017 and the next strongest was the largest pod (AJ, $p = 0.9$).

We assessed births for 94 females that were of reproductive age (10-42) at some point during the 41 years of our study; 53/97 were alive at the end of the heatwave. There were 203 documented births by these females, with births occurring between the ages of 11 and 42 yrs (Fig. 5). Most of the births occurred before the whales were in their mid-20s, and the estimated calving probability peaked around 20 years of age (Fig. 5). Although the inference about trends in fecundity was less precise than for survival (due to small numbers of births), four of the 10 index pods had evidence ($p > 0.75$) of a decline below average annual fecundity after the heatwave (two declined in 2017, three in 2018, one in 2019; Fig. 8). AB25 and AJ pods had declines in two consecutive years (2018+2019, and 2017+2018, respectively). This lagged effect is consistent with the 17-month gestation for this species, and we suggest that poor nutritional condition of females during and the heatwave was likely constraining reproduction. The strongest evidence was for AB25 pod declining in 2018 ($p = 0.96$) and the next strongest was AJ pod in 2017 ($p = 0.87$).

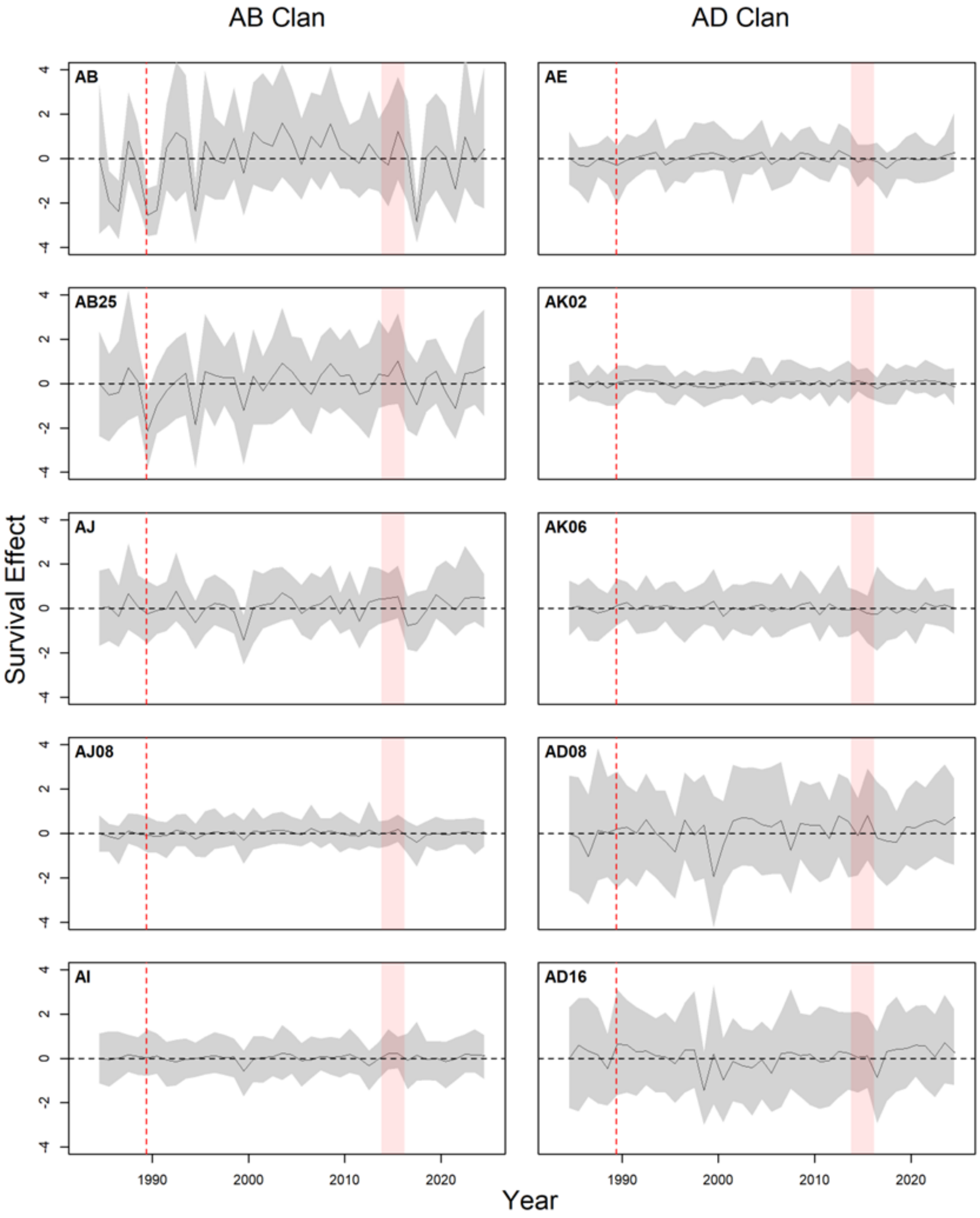

Figure 7. Estimates of annual effects to survival, indicating annual departures for each pod from the average fixed effects allowing survival to differ by age class (Table 3). Panels are included for five index pods from each of the AB and AD acoustic clans; individual pods are labelled on each. Black lines represent the most likely estimate (posterior mean) and gray error bars the 95% confidence intervals from a Bayesian mark-recapture model. The timing of the northeast Pacific marine heatwave is referenced by pink vertical shading and the Exxon Valdez oil spill by a vertical red line.

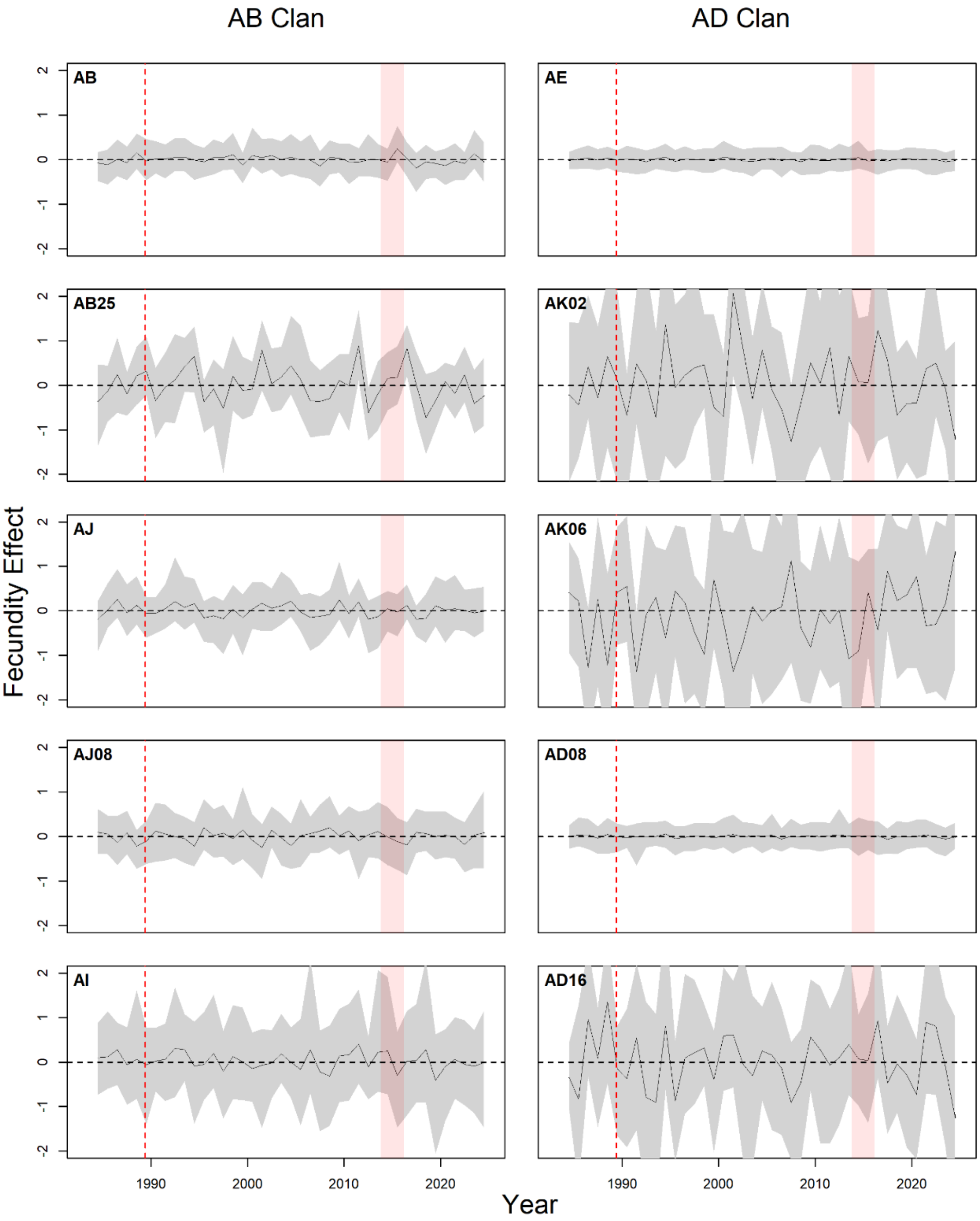

Figure 8. Estimates of annual effects to fecundity, indicating annual departures for each pod from the average fixed effects allowing the probability of calving to differ by female age (Fig. 5). Panels are included for five index pods from each of the AB and AD acoustic clans; individual pods are labelled on each. Black lines represent the most likely estimate (posterior mean) and gray error bars the 95% confidence intervals from a Bayesian model. The timing of the northeast Pacific marine heatwave is referenced by pink vertical shading Exxon Valdez oil spill by a vertical red line.

Four pods (AB, AB25, AJ and AK2) showed declines in both survival and fecundity around the heatwave, resulting in multi-year abundance declines for the AB and AJ pods, and a pause to the population growth of the AB25s and AK2s (Fig. 9). Notably, AJ8 pod, another AB clan pod, also declined in abundance, but its lower numbers and incomplete annual censuses (see error bars on Fig. 9) likely constrained power for fully diagnosing the associated demographic changes.

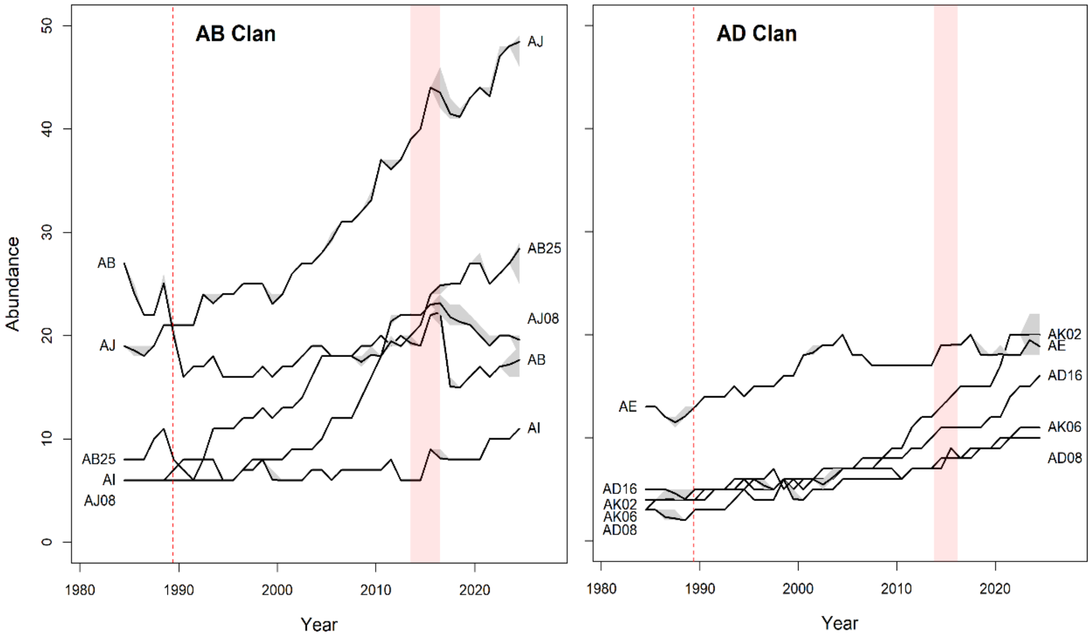

Figure 9. Number of killer whale individuals (Abundance) in each of 10 index pods that were photo-identified regularly during the 2022-2024 reporting period. The abundance of each pod is presented for the extended period 1984-2024. Black lines represent the most likely estimate (posterior mean) and gray error bars the 95% confidence intervals from a Bayesian mark-recapture model which allowed for uncertain timing of birth/death status of some individuals, where applicable. Plots include five pods from each of the AB and AD acoustic clans; pods are labelled at the start and end of abundance trajectories. The timing of the northeast Pacific marine heatwave is referenced by pink vertical shading Exxon Valdez oil spill by a vertical red line.

Incorporating photogrammetry: Changes in female length and reproductive success

During a previous reporting period, we successfully flew 20 drone flights and collected 8911 aerial images of killer whales from two of our index pods, AK02 and AD08 (Matkin et al. 2023). This demonstrated the feasibility of the approach, and we have continued in the current reporting period. In 2022, we successfully flew 65 drone flights, collecting 16,124 images from eight different resident pods including six index pods (AK02, AK06, AD08, AD16, AB25, AJ08) and in 2024 we collected 7118 images during 27 flights, documenting individuals in six different pods including five index pods (AK02, AD08, AD16, AB25, AB). Notably our aerial images included whales from index pods belonging to both the AB and AD acoustic clans.

We fit the growth curve model to 138 different annual estimates of TL for 84 different individual whales, including 29 confirmed females (Fig. 10). These ranged in age from 0.2 yrs to 45 yrs (16 to 45 yrs for confirmed females), with TLs ranging from 2.70 m to 6.17m (5.02 m to 6.17m for confirmed females). The average standard deviation across repeat measurements of the same whale in the same annual sampling period was just 6 cm (range 3 – 8 cm), evidencing the precision of our measurements. The raw data on TL provided the first evidence that older females (>30 yrs) who reached physical maturity prior to the heatwave typically had longer lengths compared to younger females (16-27 yrs; Fig. 10).

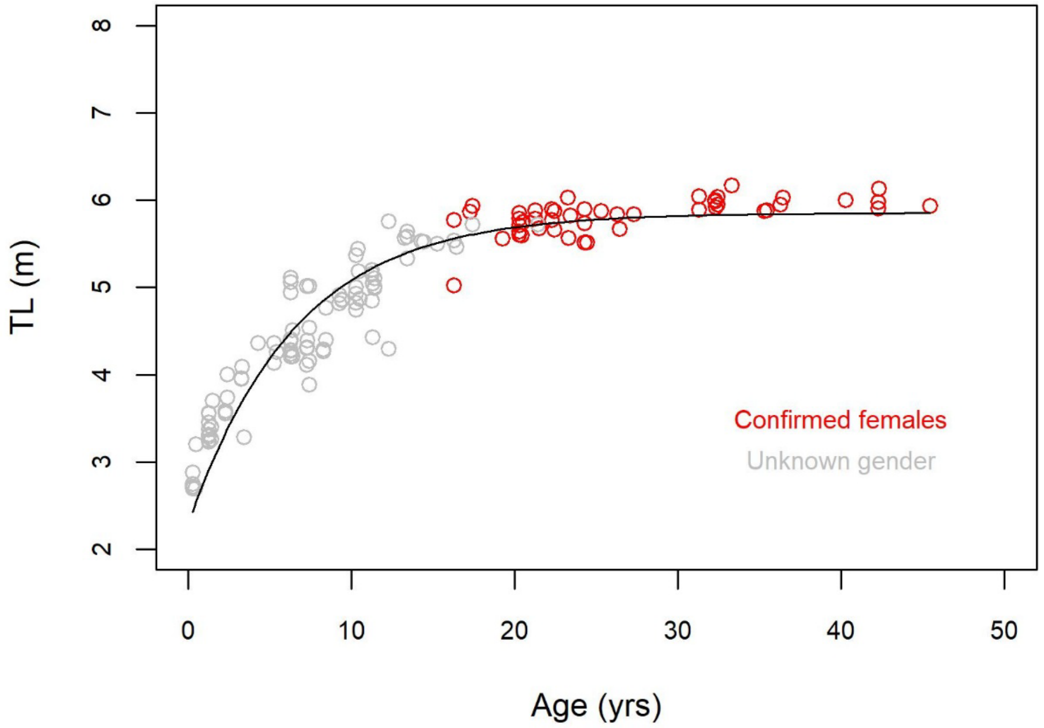

Figure 10. Estimates of total body length (TL) for killer whales of known age (84 whales, 29 confirmed females), estimated from aerial photogrammetry from an octocopter drone using a laser range finder for scale. Line indicates the posterior mean of the average asymptotic growth from a Bayesian formulation of the Richard's growth model.

To further elucidate these trends, while accounting for some repeated measurements of the same whales in different sampling periods, we analyzed the estimated asymptotic length for each whale from the growth curve model against their age at the onset of the heatwave. The fitted GAM revealed notable declines in asymptotic length for females that were growing during the heatwave (Fig. 11). This was particularly true for whales under five years of age at any point in the heatwave years (which includes those born prior to the heatwave, left of zero age on the x-axis in Fig. 11) which corresponds to when maximum growth rate occurs (see slope of growth curve on Fig. 10). Note that the entirety of the 95% confidence intervals of the GAM fit fell below the average asymptotic length for ages -3 to 5 yrs at the onset of the heatwave, implying that effects on growth persisted beyond 2016 when the heatwave began to dissipate, at least for whales born following the heatwave in 2017 and growing in subsequent years. In contrast, whales that were physically mature prior to the heatwave (ages >23 yrs at onset) and those in the latter stages of slower growth (ages ≥ 10 yrs at onset) had estimated asymptotic lengths longer than average, as did whales born five or more years after the heatwave.

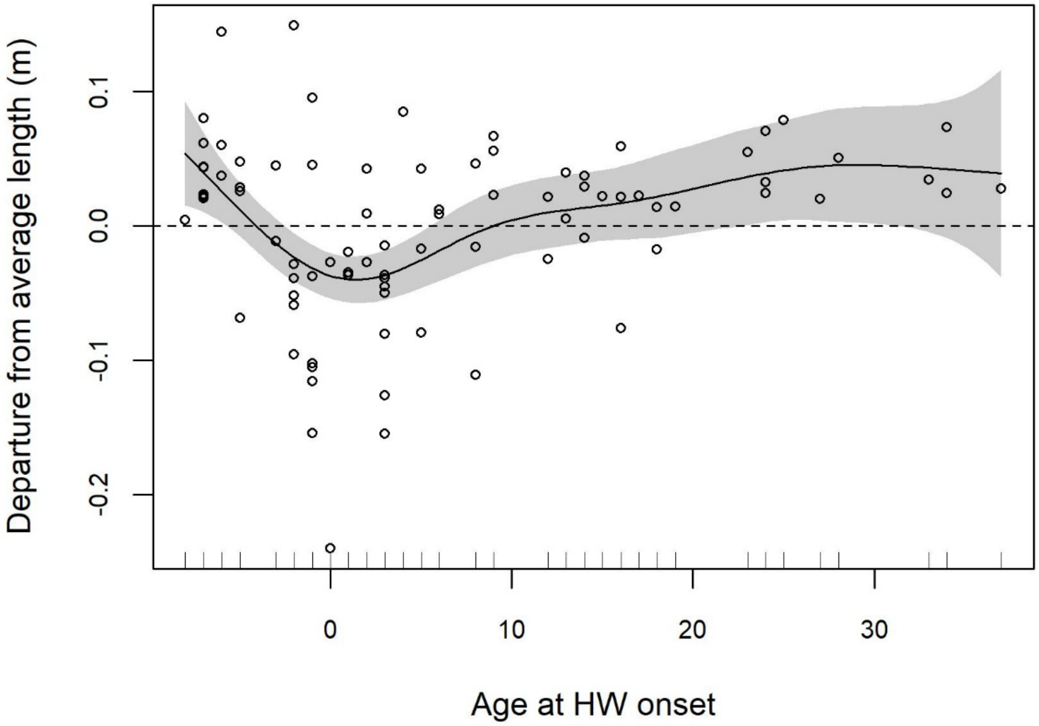

Figure 11. Fit of a generalized additive model (GAM) to the mean of female killer whales' Bayesian posterior distributions for asymptotic length to explore the shape of the relationship with their age (in years) at the onset of the Pacific marine heatwave (HW) *in early 2014. Data is presented as departure from the average asymptotic length (posterior mean of 5.84 m, here shown as dashed line). Gray ribbon shows 95% confidence intervals of the GAM fit. Negative ages are calves born after the onset of the heatwave.*

The final stage of our analysis investigated if constrained growth and ultimately smaller asymptotic size influenced fecundity. There were 27 females represented in both our measurement and fecundity datasets, responsible for 80 births between 1990 and 2024 at ages between 11 and 38 years old. By incorporating estimated asymptotic length as an individual covariate in the fecundity model, we estimated a high probability ($p = 0.98$) of a positive relationship between asymptotic length and the probability of calving, while accounting for the expected age and annual effects (Fig. 12).

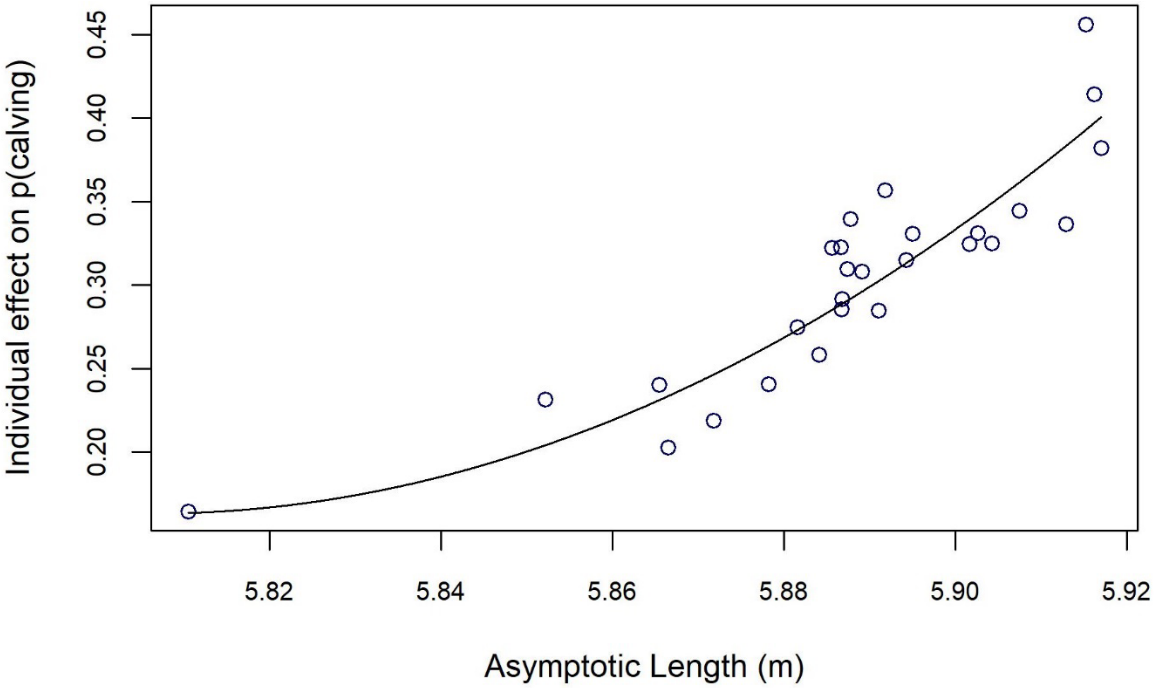

Figure 12. Individual effect on calving probability, "p(calving)", plotted against estimated asymptotic length for 27 killer whale females. Individual effect is the individual intercept that adjusts the level of the Bayesian model for calving probability against age (Fig. 5), with upward adjustments for more fecund females. This intercept term is centered on age 20 and can therefore be interpreted as their p(calving) at 20 years of age. Line is the posterior mean of linear regression model on the logit scale.

Diet

New analysis of whale fecal samples and scale samples from fish kills were not conducted due to curtailed funding, although additional samples were collected for future analyses if funding is secured. In 2022, we collected 17 free-floating killer whale fecal samples and two fish scale samples from killer whale predation events that will be genetically analyzed to identify prey species. These came from encounters with eight different resident pods. In 2023, we collected 11 fecal samples and one fish scale sample from encounters with four different pods. In 2024, we collected 10 free-floating fecal samples and two fish scale samples from encounters with seven different pods. In later analyses, these will be added to 86 fecal samples collected between 2016-2021 and 362 scale samples collected 1991-2021 (Matkin et al. 2023).

Analysis of diet from fecal and scale samples has been previously described (Matkin et al. 2023, Van Cise et al. 2024, Appendix 1).

Habitat Use

New analysis of acoustic recordings was not conducted due to curtailed funding. Analysis of year-round patterns of acoustic detections have been previously described (Myers et al. 2021, Matkin et al. 2023).

During previous research we have established acoustic monitoring stations in three areas important for resident killer whales (Hinchinbrook Entrance, Montague Strait, Resurrection Bay) to monitor changes in their distribution and habitat use (Myers et al. 2021, Matkin et al. 2023). During this reporting period, we continued to service and collect data from these bottom-mounted hydrophones and deployed new hydrophones off the northeast corner of Kodiak Island and in Kachemak Bay (Fig. 2). Monitoring across these locations will provide insight into the movement patterns and habitat preferences of killer whale pods. Analyses will be conducted once funding is secured.

We also collected field acoustic recordings from 13 different resident pods including 8/10 index pods (AB, AB25, AD05, AD08, AD11, AD16, AE, AJ, AJ08, AK02, AN10, AN20 and AX27 resident pods). These will be used to identify pod-specific dialects and describe calling rates for application to passive acoustic density estimation, when funding is secured.

DISCUSSION

Two groups of killer whales that are known to have been directly injured by the *Exxon Valdez* oil spill (Matkin et al. 2008) have not recovered to pre-spill numbers. The AT1 population numbered 22 animals prior to the spill but dropped to half of this abundance by the early 1990s. During this reported period their abundance dropped from seven to just six whales, with the loss of the youngest remaining whale. This population is headed toward extinction because the four remaining females are likely beyond reproductive ages. In contrast, the AB pod trended towards recovery in the post-spill years prior to the Pacific marine heatwave. After declining from 26 to 16 whales following the oil spill, AB pod slowly recovered to a post-spill high of 22 whales in 2015 but declined precipitously to its lowest recorded abundance of just 14 whales in 2017. AB pod has since been slowly growing, increasing to 18 at the end of this reporting period in 2024. This latest decline came at the end of a marine heatwave during 2014-2016 that has had acute and prolonged impacts on the Gulf of Alaska ecosystem (Suryan et al. 2021) and erased 30 years of post-spill recovery of AB pod (Matkin et al. 2023). This demonstrates that the impacts of environmental variation can permeate up through the marine ecosystem to these long-lived top predators and demonstrates the need for continued monitoring to understand how environmental variation will affect recovery potential.

Our understanding of the effects of climate change is relatively advanced for baleen whales that feed near the base of marine food webs and therefore display tight coupling to physical changes (Leaper et al. 2006, Braithwaite et al. 2015, Meyer-Gutbrod et al. 2021, Perryman et al. 2021, Stewart et al. 2023). However, relating physical environmental changes to impacts on marine mammals at higher trophic levels is complicated by the intervening trophic dynamics. Nonetheless there is emerging evidence of impacts from acute climatic events extending up to killer whales at the top of high-latitude marine food webs. Around the Antarctic Peninsula, poor body condition and anomalously low survival of Type B2 killer whales (penguin and likely fish predators) has coincided with recent reductions in sea ice and warmer ocean temperatures, indicating disruption to their prey populations and a decline in killer whale carrying capacity (Durban et al. 2021). We have previously shown that the encounter rate and number of animals per encounter declined for piscivorous Alaska Resident killer whales in our study area during and following the multi-year PMH (Suryan et al. 2021, Matkin et al. 2023, this report) likely a response to declines in abundance of Chinook salmon and other prey species (Van Cise et al. 2024). Here we have shown that this was accompanied by acute declines in survival of individuals in the majority of our index pods, including the strategically important AB pod and the most abundant AJ pod.

Our new analyses also inferred additional sub-lethal effects that are likely prolonged, specifically effects on reproductive success and interacting effects on female size. We used an age-structed model fit to more than four decades of calving records for known-aged females to estimate the expected fecundity-at-age relationship for Alaska Resident killer whales. We identified reductions in expected calving in the three years after the heatwave began to dissipate in 2016. This lagged effect is consistent with the 17-month gestation for this species, and we suggest that poor nutritional condition of females during and the heatwave was likely constraining reproduction. Analysis of similar reproductive data from Resident killer whale populations further south in the eastern North Pacific has revealed environmental impacts on reproduction, with correlated fecundity rates across populations (Ward et al. 2016) and strong correlation with changes in the abundance of their Chinook salmon prey (Ward et al. 2009).

Further evidence of constrained nutritional condition was gleaned from drone photogrammetry measurements of body length and patterns of growth for female Alaska Resident killer whales of known ages. Using a hierarchical Bayesian growth curve model that allowed individual variation in growth, we identified notable declines in asymptotic length for whales that were growing during the heatwave. This was particularly true for whales under five years of age when the maximum growth rate occurs, again suggesting reduced nutritional condition of young whales. Because nursing in killer whales typically continues through the first three years of life (Newsome et al. 2009), it is likely that this reduction in early growth also reflects reduced condition of their mothers which constrained lactation. Using the same aerial photogrammetry methods, we have documented changes in adult length in both Southern and Northern Resident killer whale populations that occur further south in the eastern North Pacific, with significant reductions in adult lengths of females growing during periods of lower Chinook salmon availability (Fearnbach et al. 2011, Groskreutz et al. 2019).

We estimated that the effects on early growth persisted beyond 2016 when the heatwave began to dissipate, with reduced asymptotic size estimated for whales born and growing in the years immediately following the heatwave, most notably for whales born in 2017. This is consistent with prolonged ocean warming through 2019 and prolonged impacts on lower trophic levels (Suryan et al. 2021). We hypothesize that the impacts of the PMH may be even more prolonged by an interaction between reduced female size and reproductive success. By incorporating asymptotic length as an individual covariate in the fecundity model, we estimated a high probability of a positive relationship between asymptotic length and the probability of calving, indicating that smaller body sizes confer reduced energy stores to support successful pregnancies and lactation. Using similar methods, we have documented North Atlantic right whales (*Eubalaena glacialis*) growing to smaller sizes in recent decades (Stewart et al. 2021), with shorter females having reduced fecundity (Stewart et al. 2022). We expect this effect to be greater for capital breeding baleen whales, with larger females able to amass greater energy stores during feeding seasons to support gestation and lactation during non-feeding seasons. However, reproduction is also costly for female killer whales (Fearnbach et al. 2018) and our analyses suggest that size-moderated fasting endurance is also important for this smaller odontocete species. As such, we anticipate the impacts of the PMH to be prolonged, particularly if females face the challenge of reproduction during future periods of nutritional limitation.

CONCLUSIONS

Two groups of killer whales, the AT1 population and AB pod, were impacted by the *Exxon Valdez* Oil spill, with reductions in population numbers of about 50%. This was a precipitous decline and was detected because annual monitoring of these and other killer whale groups began four years before the spill. Their lack of recovery has been monitored over four subsequent decades, with individual photo-identifications being used to track annual births and mortality events. This is arguably the most definitive and powerful study of long-term impacts from the oil spill, both on the killer whales directly and with implications for the status of the lower trophic levels in the Gulf of Alaska marine ecosystem on which these top predators depend.

Recovery of the AT1 population (mammal-eating transient ecotype) was precluded because juveniles and reproductive females did not survive the immediate years after the spill. This pod is on a trajectory to extinction; six animals remain compared to a high of 22 whales before the spill. There has been no successful reproduction since the spill and the remaining females are beyond known reproductive ages.

The AB pod (fish-eating resident ecotype) was recovering slowly following the spill, as expected given the low reproductive rate for this species. They suffered a major setback in recovery following the Pacific marine heatwave in 2014-2016, which erased 30 years of post-spill recovery and reduced the pod to just 14 whales. Acute mortality from that perturbation was precipitous, but there were also delayed declines in fecundity; both were mirrored by some other resident pods, particularly other pods in the related AB acoustic clan. AB pod, like the other pods, has resumed slow growth in the last decade, numbering 18 whales at the end of this reporting period in 2024, still below the high of 26 whales before the spill.

These population level effects have been detected because of long term population monitoring, and these data demonstrate the need for continued monitoring to understand how environmental variation will affect recovery potential. Indeed, we have revealed sub-lethal health effects from the heatwave that will likely lead to prolonged impacts on population recovery. Specifically, we have detected reduced growth of individuals during and after the heatwave, likely because of impacts on their prey species. Furthermore, we estimated a high probability of a positive relationship between asymptotic length of females and the probability of calving. Smaller body sizes confer reduced energy stores to support pregnancies and lactation. As such, the future reproductive potential of these females is reduced, particularly if they face the challenge of reproduction during future periods of nutritional limitation.

Given such clear documentation of long-term effects of the oil spill, and slow recovery that is also affected by environmental change, we strongly suggest the continued monitoring of this *Exxon Valdez* oil spill-affected species which has yet to recover to pre-spill numbers.

ACKNOWLEDGEMENTS

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APPENDIX 1

The diverse diet of southern Alaska resident killer whales changes across spatiotemporally distinct foraging aggregations

Hannah Myers[1,2]*, Daniel Olsen[3]*, Amy Van Cise[4], Kim Parsons[5], Abigail Wells[6], Craig Matkin[3]

[1]Marine Mammal Institute, Oregon State University, Newport, OR, USA
[2]College of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Fairbanks, AK, USA
[3]North Gulf Oceanic Society, Homer, AK, USA
[4] School of Aquatic and Fishery Sciences, University of Washington, Seattle, WA, USA
[5]Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, WA, USA
[6]Lynker Technologies, Leesburg, VA, USA, Under contract to Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, WA, USA

*These authors contributed equally.

Abstract

Top predators influence ecological communities in part through the prey they consume, which they often track through cycles of seasonal and geographic abundance. Killer whales are top predators in the marine ecosystem. In the North Pacific, they have diverged into three distinct lineages with different diets, of which the fish-eating type is most abundant. In this study, we examine the diet of the southern Alaska resident killer whale population across three major foraging aggregations. We take advantage of two unique sampling methods to reveal strong spatiotemporal patterns in diet from May through September. Chinook, chum, and coho salmon were each dominant in different locations and times, with substantial dietary contributions from Pacific halibut, arrowtooth flounder, and sablefish. The diverse, location-specific, and seasonal nature of the feeding habits of this marine top predator highlights the importance of diet sampling across broad spatiotemporal and population-level scales.

Introduction

Top predators influence ecological communities in part by affecting the abundance and behavior of prey species they consume. Many predators track prey resources according to their abundance, timing, ephemerality, and predictability, among other factors[1]. Both marine and terrestrial predators show pronounced seasonal patterns in foraging strategies, and individuals and family or social groups often further specialize[2–5].

Understanding changes in diet across time, space, and among conspecifics is therefore critical to assess the ecological effects of predators across ecosystems.

Killer whales (*Orcinus orca*) are top predators in the marine ecosystem and are most abundant in high-latitude regions[6]. In the North Pacific, they have diverged into three distinct ecotypes that may represent separate species[7]: those that eat exclusively fish (known as residents), those that eat exclusively mammals (called transients or Bigg's), and those that likely consume mostly sharks (known as offshores)[7,8]. The fish-eating type is most abundant, with at least four studied parapatric populations spanning coastal regions in the northeast Pacific[8]. The 75 animals in the critically endangered southern resident population are found primarily in the Salish Sea and off the outer Washington coast[9]. The northern resident population of more than 300 individuals is found primarily off the coasts of British Columbia and southeast Alaska[10]. The southern Alaska resident population of about 1,000 killer whales ranges from southeast Alaska to Kodiak Island[11]. Finally, roughly 1,000 animals in the western Alaska North Pacific population range from Kodiak Island into the Bering Sea[12].

Pacific salmon (*Oncorhynchus spp.*) are important prey for all studied North Pacific fish-eating killer whale populations. Southern residents, in particular, feed almost exclusively on Chinook salmon (*Oncorhynchus tshawytscha*) in spring and summer—though their diet is significantly more diverse in fall and winter[13]. Northern residents forage mostly for Chinook salmon and chum salmon (*Oncorhynchus keta*)[14] in summer. Southern Alaska residents eat substantial portions of Chinook, chum, and coho salmon (*Oncorhynchus kisutch*)[15,16]. Western Alaska North Pacific residents are less well-studied, but may consume lower trophic level fish further west where salmon are less available[17], and at least some pods depredate on groundfish species[18]. On the other side of the Pacific, fish-eating killer whales in Avacha Gulf eat mostly coho and chum salmon in summer[19].

While the importance of salmon as prey for North Pacific fish-eating killer whales is well-established, how these animals utilize different prey resources across space and time is largely undocumented—especially for the substantially larger populations found off Alaska. In this study, we take advantage of a long-term killer whale monitoring program to examine how the diet of southern Alaska resident killer whales changes across three main foraging aggregations. We use both prey (scale and flesh) samples and fecal samples to identify the relative importance of different prey species. This work adds to our understanding of prey selection by fish-eating killer whales, providing valuable insights into their spatiotemporal foraging strategies and the potential ecological impacts of prey variability.

Results

We collected 255 samples of fish scales or flesh while southern Alaska resident killer whales were observed feeding across 31 years (1991 to 2021). We collected 186 fecal samples across six years (2016 – 2021), of which 87 successfully sequenced, were not from the same individual on the same day, and passed the quality check. These samples were collected in three adjacent geographic areas with largely non-overlapping data collection periods: Kenai Fjords from mid-May to mid-June, eastern Prince William Sound from mid-June to July, and western Prince William Sound from July through September (Figure 1). Both prey (scale and flesh) and fecal samples demonstrated distinct dietary patterns across these three foraging aggregations. Chinook and chum salmon were the dominant species across all diet samples, although coho salmon were the most common prey sample collected in western Prince William Sound. Fecal samples also revealed substantial contributions from Pacific halibut (*Hippoglossus stenolepis*), arrowtooth flounder (*Atheresthes stomias*), and sablefish (*Anoplopoma fimbria*).

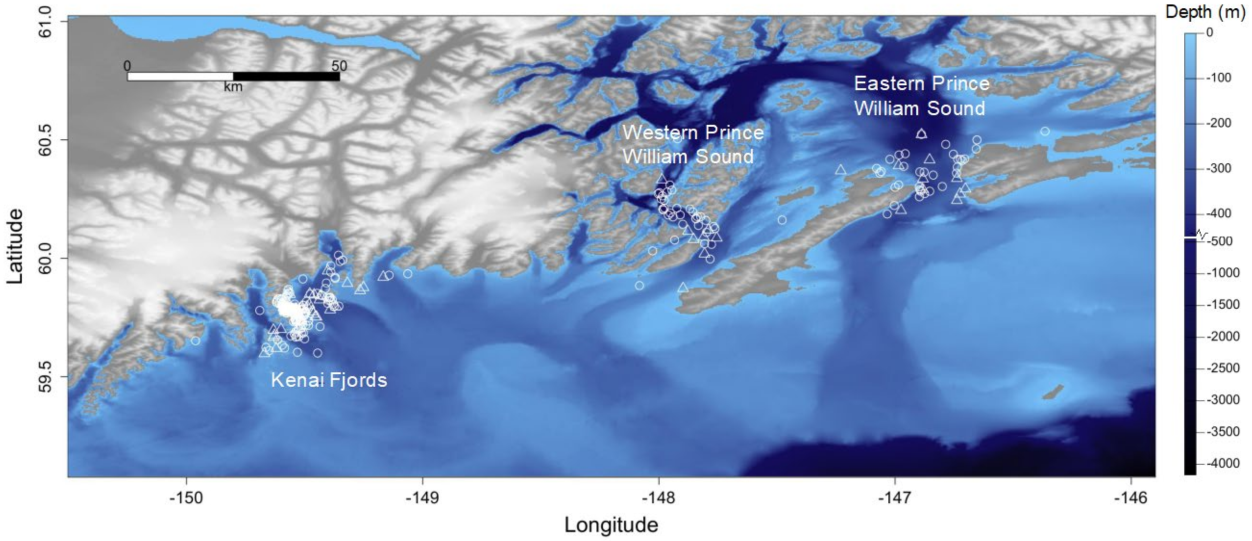

Figure 1. Map of prey (circle) and fecal (triangle) samples collected in the study area in the northern Gulf of Alaska.

Prey samples

Of the 255 scale and flesh samples we collected, 249 were of three salmon species: Chinook, chum, and coho (Table 1, Figure 2). Only one prey sample did not clearly align with an aggregation area and was therefore removed from the analysis (chum salmon collected September 4th, 2020 at 59.788° N, 148.705° W). Chinook salmon made up 77% of prey samples collected in Kenai Fjords—where 80% of samples were collected between May 17th and June 12th. Chum salmon were the primary prey species in eastern Prince William Sound (62% of samples), where sampling took place primarily from June 15th to July 22nd. Coho salmon dominated the western Prince William Sound foraging aggregation (77%), where we collected samples primarily from July 22nd to September 10th. In addition to the three main salmon prey species, five sockeye salmon (*Oncorhynchus nerka*) prey samples were collected in May and June in Kenai Fjords and eastern Prince William Sound, and one Pacific herring (*Clupea pallasii*) sample was collected in early May in Kenai Fjords.

The main pods (maternally related family groups) differed across the three foraging aggregations, although samples were collected from the AK pod across all three (Table 1).

Table 1. Fish scale and flesh samples from three primary salmon prey species, collected while killer whales were observed feeding. The period within which 80% of prey samples were collected and the range of dates within which all prey samples were collected are shown for each area. The main pods are those from which at least 5% of samples were collected, with the number of samples from that pod in parentheses. The predominant prey species from each foraging aggregation is shown in bold.

Area	Total samples	Sampling period (80% of samples / all samples)	Main pods (*n*)	Salmon species	Proportion of samples
Kenai Fjords	149	May 17 – Jun 12 / May 3 – Sep 8	AD8 (41), AK (37), AD5 (24), AD16 (16)	**Chinook**	**.78**
				Chum	.19
				Coho	.03
Eastern Prince William Sound	37	Jun 15 – Jul 21 / Jun 14 – Aug 29	AB (7), AD8 (4), AE (4), AJ (4), AK (4), AI (3), AX48 (2)	Chinook	.16
				Chum	**.62**
				Coho	.22
Western Prince William Sound	62	Jul 22 – Sep 9 / Apr 29 – Sep 21	AE (26), AB (9), AK (8), AI (4)	Chinook	.19
				Chum	.03
				Coho	**.77**

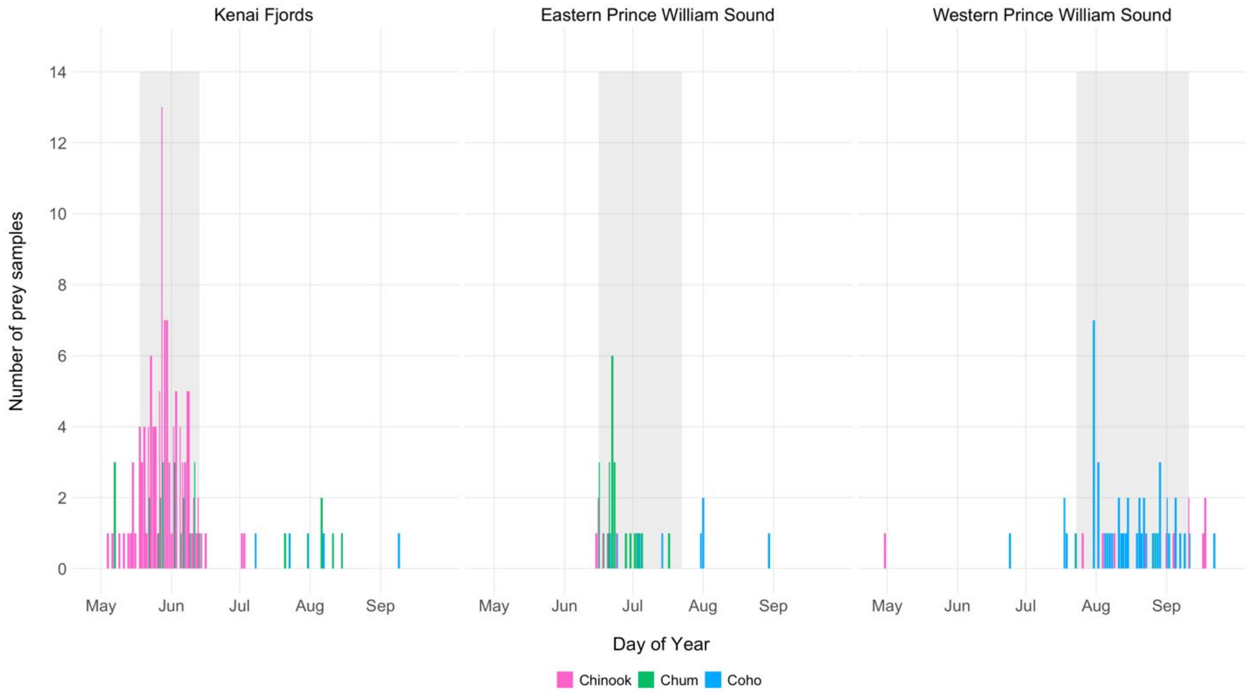

Figure 2. Prey samples collected from each area from May through September across all years. Gray shaded periods indicate the days in which 80% of prey samples were collected in each area.

We used a Bayesian multinomial logistic regression model to test for relationships between prey species and foraging aggregation. Model results reflected the strong diet patterns described above (Table 2). Coefficients for which the 95% credible interval does not include zero may be interpreted as statistically significant. In particular, the strong quadratic effect for chum salmon reflects the high probability of chum salmon in eastern Prince William Sound, and the strong linear effect for coho salmon reflects the higher probability of coho salmon in western Prince William Sound. Posterior predictive checks indicated good model fit to the data and model convergence and $\hat{R}$ was 1.00 for all parameters.

Table 2. Prey sample multinomial logistic regression model results for the probability of Chinook, chum, and coho salmon prey as a function of aggregation area. The model reference level is Chinook salmon. The linear term describes the relationship across the three ordered areas: Kenai Fjords, eastern Prince William Sound, and western Prince William Sound. The quadratic term reflects how the middle level (eastern Prince William Sound) differs from the expected linear relationship between the first (Kenai Fjords) and last (western Prince William Sound) levels. Statistically significant effects (those for which the 95% credible interval does not include zero) are shown in bold.

Species	Coefficient	Estimate	Estimated error	Lower 95% credible interval	Upper 95% credible interval
Chum salmon	Intercept	**-0.66**	**0.33**	**-1.36**	**-0.06**
	Linear	-0.39	0.60	-1.72	0.65
	Quadratic	**-2.55**	**0.53**	**-3.66**	**-1.59**
Coho salmon	Intercept	-0.49	0.27	-1.03	0.04
	Linear	**3.28**	**0.40**	**2.55**	**4.12**
	Quadratic	-0.99	0.51	-2.01	0.01

Fecal samples

Results from 87 fecal samples reinforced the primary importance of salmonids in the diet of southern Alaska resident killer whales while also revealing three major prey items that are presumably captured and consumed at depth: Pacific halibut, arrowtooth flounder, and sablefish (Table 3, Figure 3). As with prey samples, Chinook salmon was the dominant species in fecal samples from Kenai Fjords (71%) and chum salmon was the dominant species detected in eastern Prince William Sound (72%). In western Prince William Sound, diet samples were substantially more diverse, with the greatest contribution from Chinook salmon (35%). The main sampling periods largely aligned for both prey and fecal samples, however, fecal samples were collected in fewer years and sample sizes were notably lower, especially from Prince William Sound (Tables 1 and 3).

Table 3. Fecal samples from six main fish prey species. The period within which 80% of fecal samples were collected and the range of dates within which all fecal samples were collected are shown for each area. The species with the highest overall proportion from each foraging aggregation is shown in bold.

Area	Total samples	Sampling period (80% of samples / all samples)	Pods identified (*n*)	Major prey species	Proportion across all samples
Kenai Fjords	66	May 24 – Jun 8 / May 17 – Sep 17	AD8 (21), AK (18), AD16 (10), AJ (1), AX48 (1)	**Chinook**	**.71**
				Chum	.26
				Coho	.00
				Halibut	.03
				Arrowtooth	.00
				Sablefish	.00
Eastern Prince William Sound	12	Jun 15 – Jun 30 / May 9 – Jul 1	AK (3), AE (1), AJ (1), AX48 (1)	Chinook	.04
				Chum	**.72**
				Coho	.02
				Halibut	.07
				Arrowtooth	.13
				Sablefish	.03
Western Prince William Sound	9	Jul 3 – Sep 30 / Jul 2 – Sep 30	AK (2), AB25 (1), AE (1), AI (1), AJ (1)	**Chinook**	**.35**
				Chum	.20
				Coho	.12
				Halibut	.24
				Arrowtooth	.09
				Sablefish	.00

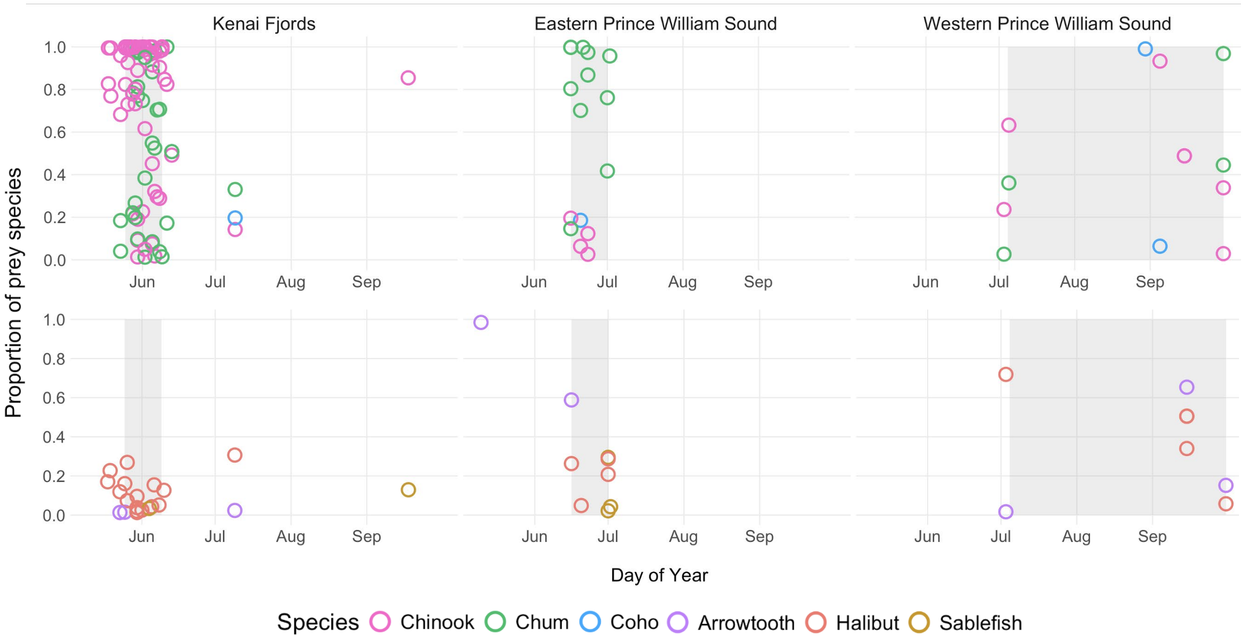

Figure 3. Proportion of prey species in fecal samples collected in each area from May through September. Chinook, chum, and coho salmon are in the top panel and Pacific halibut, arrowtooth flounder, and sablefish are in the bottom panel. Gray shaded periods indicate the days in which 80% of fecal samples were collected in each area.

Host DNA from 48 unique whales was identifiable in 82 samples and samples originating from the same host identified. Pod identity was assigned in 63 cases based on photo-identification at the time of sampling (Table 3). As with prey samples, fecal samples were collected from the AK pod across all three foraging aggregations, though there were differences in some of the other pods sampled across events.

Halibut was detected in all regions and made up >5% of at least one sample in May, June, July, and September. It was consumed regularly by the AE, AK, AD8, and AD16 pods. Arrowtooth flounder was detected in the greatest proportions in May, June, and September, primarily in samples from the AE pod. In addition to the six major prey species, three fecal samples contained 1-3% prowfish (*Zaprora silenus*), all of which were from Kenai Fjords.

For fecal samples, we fit a Bayesian multinomial model with a Dirichlet response distribution to describe the effect of foraging aggregation on the probabilities of the six main prey species (Table 4). The strongest effects were evident for chum and Chinook salmon: the high proportion of chum salmon in eastern Prince William Sound (represented by the intercept) and the high proportion of Chinook salmon in Kenai Fjords were statistically significant (i.e., the 95% credible intervals did not include zero). Flatfish were more likely in western Prince William Sound, though not significantly so. Other species' proportions varied but effects were not consistently significant. Posterior predictive checks indicated sufficient model fit to the data and model convergence and $\hat{R}$ was 1.00 for all parameters.

Table 4. Fecal sample model results for the probability of six main prey species as a function of foraging aggregation. Sablefish is the reference species and eastern Prince William Sound is the reference area against which intercepts and coefficients for Kenai Fjords (KF) and western Prince William Sound (WPWS) are compared. Statistically significant effects (those for which the 95% credible interval does not include zero) are shown in bold.

Species	Coefficient	Estimate	Estimated error	Lower 95% credible interval	Upper 95% credible interval
Arrowtooth flounder	Intercept	-0.15	0.42	-1.00	0.65
	KF	0.29	0.45	-0.61	1.18
	WPWS	0.77	0.64	-0.52	2.01
Pacific halibut	Intercept	0.28	0.41	-0.55	1.09
	KF	0.21	0.45	-0.67	1.09
	WPWS	0.81	0.62	-0.41	2.02
Chum salmon	Intercept	**1.95**	**0.35**	**1.28**	**2.67**
	KF	**-1.20**	**0.38**	**-1.97**	**-0.46**
	WPWS	**-1.34**	**0.58**	**-2.49**	**-0.25**
Coho salmon	Intercept	-0.20	0.41	-1.02	0.61
	KF	0.17	0.44	-0.71	1.04
	WPWS	0.44	0.63	-0.80	1.67
Chinook salmon	Intercept	0.47	0.42	-0.34	1.31
	KF	**1.76**	**0.44**	**0.88**	**2.64**
	WPWS	1.17	0.60	-0.01	2.35

Additional observations

The rich data available from fecal samples revealed several unique observations that complement the data generated from prey scales and tissue remains. First, fecal samples were collected across multiple foraging aggregations from four individual whales, all from the AK pod. Consistent with overall results, samples from these individuals included mostly Chinook salmon during the Kenai Fjords foraging aggregation and mostly chum salmon during the eastern Prince William Sound aggregation. Second, on May 29th, 2018, fecal samples were collected from both adult female AD31 and her 4-year-old male offspring AD54, in the AD8 pod. Samples from both mother and offspring contained the same four species in very similar percentages: 81% and 77% chum salmon, respectively; 9% and 19% Chinook salmon, 9% and 4% Pacific halibut, and <1% each arrowtooth flounder. This unique collection event highlights an instance of high diet similarity between a known mother and dependent offspring—a pair of animals likely to share prey[20]. Third, in 2018, we observed a change in foraging patterns with killer whales foraging farther offshore than typical in Kenai Fjords. We documented a notably higher proportion of chum salmon (76%) in the 14 fecal samples collected in Kenai Fjords that year.

Discussion

In this study, we found that southern Alaska resident killer whales utilized different prey resources across spatiotemporally distinct foraging aggregations. The shift from primarily Chinook to chum to coho salmon across these aggregations likely reflects the relative availability of these three priority prey resources. We also found that, even from May to September when salmon are abundant, southern Alaska resident killer whales supplement their diet with other high-energy-content fishes—particularly Pacific halibut, arrowtooth flounder, and sablefish.

In general, the strong agreement between prey and fecal samples in terms of primary species, timing, and pods involved reinforced the consistency of these killer whale foraging aggregations. A notable exception is the prevalence of coho salmon in prey samples from the western Prince William Sound foraging aggregation (77%) relative to its proportion in fecal samples from that area (12%). The low number of fecal samples from western Prince William Sound (*n* = 9) makes interpretation challenging; however, most prey samples were collected in late July and early August (Figure 2), whereas no fecal samples were collected during that time (Figure 3). In addition, 80% of prey samples from western Prince William Sound were collected prior to 2008, while all fecal samples were collected after 2016, so changes in prey availability or preferences over time may also be relevant. While interannual differences in killer whale diet were not specifically investigated as part of this study, the disproportionately high level of chum salmon detected in Kenai Fjords in 2018 suggests that they are likely. Although few fecal samples were collected in eastern Prince William Sound, the species composition was closely aligned for fecal (*n* = 12) and prey (*n* = 37) samples from that foraging aggregation.

Pacific salmon are an abundant yet locally ephemeral resource in this region. Tracking the spatiotemporal variation in their phenology—including across species—extends foraging opportunities on this important prey resource for consumers. For example, the distribution of coastal brown bears (*Urus* arctos) and glaucous-winged gulls (*Larus glaucescens*) reflects the shifting distribution of spawning sockeye salmon (*Oncorhynchus nerka*) within a watershed[21]. Individual brown bears visit multiple salmon spawning sites in synchrony with their spawning phenology[22]—and those that track spawning phenology for the longest consume the most salmon[4]. Though the pulsed nature of salmon availability in the marine environment is less well-defined, the results of this study suggest that fish-eating killer whales may also capitalize on changes in Pacific salmon availability across relatively short spatial and temporal scales.

Populations with diverse diets may include specialized individuals or groups that feed on a restricted subset of prey[23]. The distinct prey patterns we documented may reflect killer whales tracking prey resources as well as different prey preferences among pods, which may be culturally transmitted[24]. The main pods detected in this study generally have different core use areas[25] and differ genetically[26]. The Kenai Fjords aggregation was dominated by pods that have a maternal haplotype shared with southern resident killer whales, while the pods encountered in eastern Prince William Sound mostly have a haplotype shared with the northern residents, and the pods commonly encountered in western Prince William Sound are more mixed. We found much higher proportions of flatfish in samples from the AE pod, which may indicate a slight degree of specialization for that pod. Almost all samples that included flatfish were from pods with the southern resident haplotype, though few fecal samples for which pod was identified were of the northern resident haplotype (*n* = 4). In contrast, the fecal samples from the AK pod matched the overall trend of high proportions of Chinook salmon during the Kenai Fjords aggregation and high chum salmon during the eastern Prince William Sound aggregation. On a finer scale, foraging strategies differ among sex- and reproductive classes of southern and northern resident killer whales[27]. While not a focus of this study, these classes are worthy of investigation.

The shifts in diet we documented for southern Alaska resident killer whales occurred within a limited season (May to September). However, seasonal changes in diet throughout the year are also common among top predators—even those with highly specialized foraging strategies. For example, wolves (*Canis lupus*) on Yellowstone National Park's northern range whose diets are dominated by elk (*Cervus elaphus*) hunt more mule deer (*Odocoileus hemionus*) and bison calves (*Bison* bison) in spring and summer. In Alaska, some wolves that hunt ungulates also eat large portions of Pacific salmon in summer[3]. Migrating gray whale (*Eschrictius* robustus) calves traveling through a geographic pinch point into the Bering Sea provide a pulse of high-quality prey that attracts mammal-eating killer whales in spring[2]. These killer whales change to a diet of largely northern fur seals (*Callorhinus ursinus*) in summer[28]. As previously mentioned, southern resident killer whales eat almost exclusively Chinook salmon in spring and summer, but in fall and winter up to about half of their diet is made up of chum and coho salmon, steelhead (*Oncorhynchus mykiss*), lingcod (*Ophiodon elongatus*), big skate (*Rana binoculata*), and flatfishes[13].

In the northeast Pacific, fish-eating killer whale diet sampling has been highly biased to summer months and shallow coastal regions where salmon are likely to be abundant—including in this study[14,16,29]. At present, southern Alaska residents' primary prey resources for more than half the year (October through April) remain unknown. Our current research effort likely misses other major foraging aggregations, even during the field season. The southern Alaska resident population is acoustically detected most often and with the largest estimated group sizes in late fall and winter in western Prince William Sound, and the peak in use of eastern Prince William Sound begins in early spring[30,31]—periods when no diet sampling took place. Lower quality or more difficult to access prey (such as flatfish) may be disproportionately important to killer whales if the their phenological patterns mean that they are available when higher quality prey (such as Chinook salmon) are not[1]. However, abundant but notably smaller and lower calorie fishes—especially pink salmon (*Oncorhynchus gorbuscha*) and herring—were not detected in proportions >1% in any fecal sample in this study. It is possible that the small proportion of prowfish found in fecal samples and the single herring prey sample we collected were secondary prey (i.e., a fish eaten by killer whale prey). Prowfish are a documented prey item of Pacific halibut, but not of Pacific salmon, arrowtooth flounder, or sablefish[32,33].

Similarly, pooling diet samples without accounting for spatiotemporal differences in sampling effort may mask important patterns[34]. For example, in this study, 59% of prey samples and 76% of fecal samples were collected in Kenai Fjords. Although sampling in Kenai Fjords took place for the shortest period of time, the protected waters in this area created better conditions to search for samples. The smaller killer whale group sizes encountered in this location enabled faster photo-identification, allowing more search time for diet samples. Our diet data is therefore overweighted with samples that contain a high proportion of Chinook salmon from this relatively short foraging aggregation, and if samples were not separated by aggregation the importance of chum and coho salmon at other places and times would be diluted. Additionally, collecting fish scales or pieces of flesh when killer whales are observed feeding at the surface biases results toward salmonids, while species that are captured and consumed at depth (and those without scales) go undetected. DNA analysis of fecal samples has revealed a greater diversity of fish species consumed by resident killer whales[13,15,35].

Assumptions about the diets of predator populations are frequently drawn from data collected over brief periods in specific regions, often focusing on unique (sub)populations that may not represent broader spatiotemporal or species-level scales. Samples collected in summer in the Salish Sea from southern resident killer whales are overrepresented in North Pacific fish-eating killer whale diet studies[36–38]. Extrapolating the diet of this endangered population across three populations several orders of magnitude more abundant may lead to problematic conclusions with potential management implications[36–38]. This study adds a robust analysis to the growing body of diet research from other fish-eating killer whale populations in the North Pacific[15,19,39].

Finally, predator populations with highly specialized diets are likely to be more vulnerable to disturbance—including climate change impacts—than more generalist predators with greater flexibility in their diets[40,41]. We found that southern Alaska resident killer whales utilized three different primary salmonid prey resources across three main summer foraging aggregations, with substantial supplementation from other fishes. This suggests that the southern Alaska resident population—which is thought to be growing at a rate near maximum[11]—has a more diverse summer diet than that of the endangered southern resident population[13]. Specializing on very high trophic level fish (e.g., Chinook salmon) is also likely to limit population growth compared to eating a more diverse diet that includes relatively lower trophic level fishes. However, whether the diet of southern Alaska resident killer whales remains more diverse year-round has yet to be studied.

Methods

Sample collection

Fieldwork took place between May and September in Prince William Sound and Kenai Fjords, Alaska (Figure 1). Prey samples were collected from 1991 to 2021 and fecal samples were collected from 2016 to 2021. Prince William Sound is characterized by large barrier islands, entrances with strong tidal currents, and small islands scattered near glacially carved trenches. Kenai Fjords runs along the southern Alaska coast and has many long glacially carved fjords. All fieldwork was conducted from a 11 m research vessel concurrent with other research tasks including photo-identification, acoustic research, and body condition assessment.

Prey samples from predation events and fecal samples were collected from surface waters during focal follows of resident killer whale groups. Predation events were often identified when killer whales made tight turns at the surface and/or when a whale approached to share fish with its mother. Scales or tissue samples were typically found in upwellings (upward currents from the flukes of a diving whale) after observing that a fish had been shared or broken apart at the surface. Fecal samples were usually found in upwellings while the vessel was travelling >100 m behind the whales. Samples were collected from the bow using a long-handled fine-mesh dip net and transferred into new glass jars. All samples were labelled and frozen within 10 min of collection; prey samples were frozen in ethanol.

The individuals, pods, and number of killer whales involved in each encounter in which a diet sample was collected was determined using photo-identification. Identification photos were taken with a Nikon D700 or D750 camera with a 300 or 400 mm lens and matched to a long-term photo-identification catalog maintained by the North Gulf Oceanic Society[42]. In each encounter, all members of a matriline (defined as a mother or grandmother and her offspring) were assumed to be present if at least one member of the matriline was photographed, as in Olsen *et al.*[43] and Myers *et al.*[44].

Prey species identification

Prey samples were identified to species using fish scale morphology or genetic analysis, as in Withler *et al.*[45], Ford and Ellis[14], Hanson *et al.*[29]. Only one sample per hour was retained for analysis to avoid pseudoreplication. For fecal samples, prey species composition and host identity were determined using the genetic techniques described below.

Fecal sample sequencing

Whole genomic DNA was extracted from a pea-sized subsample of frozen fecal matter using the QIAamp® Fast DNA Stool mini kit following standard protocols executed using the QIAcube automated extraction robot[35]. 16S SSU rDNA was targeted using custom-designed Illumina primers for salmon and groundfish, as previously published for the prey metabarcoding of southern resident killer whales[35]. Amplification reactions contained 4uL of DNA, 1X Promega GoTaq Flexi buffer (Promega Corp., Madison WI), 3.0mM MgCl2, 0.2mM of each dNTP, 0.1ug/uL of BSA, 0.2uM of each primer, and 2 units of Promega GoTaq Flexi DNA Polymerase. Communities were amplified in a 32-cycle PCR, with cycling conditions as follows: initial denaturation at 94°C for 2 min, followed by 32 cycles of 94°C for 35 sec; 61°C for 1 min; 72°C for 35 sec; and a final extension at 72°C for 5 min. Amplicons were gel cleaned using Qiagen MinElute columns to remove non-target PCR products and primer dimer.

Cleaned amplicons were individually indexed using two different sets of indices. In 2018 and 2019, Illumina Nextera forward and reverse index tags were used, which create a unique combination of indices by using a unique forward primer for each column and a unique reverse primer for each row on a 96-well plate. This indexing PCR was completed using a 50uL reaction containing 8uL of gel purified PCR product, 1X NEB Phusion High-Fidelity master mix (New England BioLabs), 0.2mM of each dNTP, 5uL each of one Illumina Nextera forward and reverse index tag. In the two 2021 MiSeq runs , Illumina Nextera DNA Unique Dual Index (UDI) primers were used (Illumina, Inc.), comprising unique forward and reverse indexes for each well in a 96-well plate in order to reduce the effect of index hopping[46]. The index PCR was performed in a 40ul reaction containing 8uL of gel purified PCR product, 1uL each of one Illumina Nextera UDI forward and reverse index, and 30uL of Phusion High-Fidelity PCR Master Mix (New England Biolabs). Regardless of the indices used, the indexing PCR conditions remained the same: 72°C for 3 min, 98°C for 30 sec, followed by 12 cycles of 98°C for 10 sec, 55°C for 30 sec, 72°C for 30 sec, and a final extension at 72°C for 5 min. Samples were sequenced on four sequencing runs (2018, 2019, and two in 2021) using an Illumina MiSeq next generation sequencer (Illumina, Inc.) at the Northwest Fisheries Science Center, NOAA Fisheries, Seattle, WA.

In addition to fecal samples from southern Alaska resident killer whales, two mock communities including pre-determined quantities of genomic DNA from several vouchered fish species were included on each of the four sequencing runs to detect and control for any potential bias caused by index hopping, species-specific amplification efficiency, or genotyping error. Details on mock community generation can be found in Van Cise *et al.*[15]. Mock communities were sequenced alongside sample libraries each year.

Fecal sample sequence alignment and QAQC

Sequences from all runs were combined and analyzed using a custom pipeline based on the dada2 package[47] in the R computing environment[48]. This pipeline includes steps for (1) trimming sequences based on general sequence quality, (2) filtering sequences based on a maximum number of expected errors[49], (3) learning the error rates for each possible transition, (4) de-replicating sequences by combining and counting identical sequencing reads to reduce computation time, and finally inferring unique amplicon sequence variants (ASVs) from the filtered and trimmed sequences using the previously learned error rates[47]. Once unique ASVs were identified, paired forward and reverse reads were merged and chimeras removed. Taxonomy was assigned to the remaining ASVs using a naïve Bayesian classifier[50] that relies on a fasta-formatted reference database, which we custom-generated by downloading sequences for all fish and shark species from NCBI GenBank.

Because various sources of laboratory-introduced bias can affect the observed number of reads assigned to a given species, mock community control samples were used to estimate and correct for the effects of errors, e.g. from amplification bias and index hopping. This model was run using both mock communities to estimate species-specific bias and read proportions by species were corrected based on model estimates (Figure 2, Van Cise *et al.*[15]). Some prey species in the final dataset were not anticipated, e.g. sablefish, and therefore not included in the mock communities and could not be corrected. However, overall differences between uncorrected and corrected proportional data are small enough that the difference for these species is expected to be minor (Supplemental Figures S2 and S3, Van Cise *et al.*[15]).

Final data filtering consisted of removing ASVs that assigned to *Orcinus orca* and aggregating ASVs by species. Laboratory and field duplicates used to track potential sources of bias or contamination were removed from the dataset before analysis. Samples with a read depth < 25,000 reads were removed from the dataset. Additionally, individual whale ID was genetically determined using a previously developed panel of SNPs (see Van Cise *et al*[15]. for methodological details), and samples were removed if they were collected from the same individual on the same day to avoid pseudoreplication. Prey species were only included in downstream analyses if they represented >1% of the reads in one or more samples in the dataset to avoid potential bias from genotyping error.

Statistical analyses

We modeled the effect of foraging aggregation on prey species composition with Bayesian multinomial regression models. For prey samples we used a multinomial logistic regression, for fecal samples we used a Dirichlet-multinomial distribution to handle the proportional nature of the data. We used the area in which the sample was collected (Kenai Fjords, eastern Prince William Sound, or western Prince William Sound) as a three-factor categorical variable to represent the foraging aggregation. Models were implemented using the *brms* package[51] in R (version 4.3.3) with the default uninformative priors.

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