FORAGING ECOLOGY OF SEXUALLY-DIMORPHIC MARINE GENERALIST PREDATORS: DESCRIBING STELLER SEA LION DIET ALONG THE NORTHERN WASHINGTON COAST

By

Zoë Kathryn Lewis

Accepted in Partial Completion
of the Requirements for the Degree
Master of Science

ADVISORY COMMITTEE

Dr. Alejandro Acevedo-Gutiérrez, Co-chair

Dr. Dietmar Schwarz, Co-chair

Ms. Adrianne Akmajian,

GRADUATE SCHOOL

Dr. David L. Patrick, Dean

Master's Thesis

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Western Washington University, I grant to Western Washington University the non-exclusive royalty-free right to archive, reproduce, distribute, and display the thesis in any and all forms, including electronic format, via any digital library mechanisms maintained by WWU.

I represent and warrant this is my original work, and does not infringe or violate any rights of others. I warrant that I have obtained written permissions from the owner of any third party copyrighted material included in these files.

I acknowledge that I retain ownership rights to the copyright of this work, including but not limited to the right to use all or part of this work in future works, such as articles or books.

Library users are granted permission for individual, research and non-commercial reproduction of this work for educational purposes only. Any further digital posting of this document requires specific permission from the author.

Any copying or publication of this thesis for commercial purposes, or for financial gain, is not allowed without my written permission.

Zoë K. Lewis

8/05/22

FORAGING ECOLOGY OF SEXUALLY-DIMORPHIC MARINE GENERALIST PREDATORS: DESCRIBING STELLER SEA LION DIET ALONG THE NORTHERN WASHINGTON COAST

A Thesis
Presented to
The Faculty of
Western Washington University

In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

by
Zoë Kathryn Lewis

ABSTRACT

Understanding generalist predator impact on prey populations requires an understanding of predator diet composition, foraging ecology and specialization, all of which may vary over spatial and temporal scales. Steller sea lions (*Eumetopias jubatus)* are large, sexually dimorphic, generalist predators that may have different roles in the ecosystem based on sex. However, the variation between individuals within a population, or intrapopulation feeding diversity of Steller sea lions has not been examined. In this study, I describe the diet of Steller sea lions along the northern coast of Washington between December 2020-August 2021 using DNA metabarcoding, hard parts analysis, and qPCR sex determination to examine diet composition and factors influencing intrapopulation feeding diversity. I found that the diet composition of Steller sea lions along the northwest Washington coast from December 2020-August 2021 was mainly comprised of American shad (*Alosa sapidissima*), Pacific herring (*Clupea pallasii*), big skate (*Raja binoculata)*, walleye pollock (*Gadus chalcogrammus*) and starry flounder (*Platichthys stellatus*). I found that intrapopulation feeding diversity, a proxy for individual specialization, is not influenced by season and sex. Further, individuals that exhibited generalist foraging techniques correlated with pelagic prey items such as American shad, Pacific herring, and Pacific salmon (*Oncorhynchus* spp.), which suggest that Steller sea lions in this region generally exhibit pelagic foraging techniques resulting in consumption of species of conservation concern.

ACKNOWLEDGEMENTS

Scat collections were conducted by the Makah Tribe under a General Authorization Research Permit (LOC No. 19430) and this research was funded by the Makah Tribe through the Washington Sea Grant 55151. Thank you to the Makah Tribe for the support and allowing me to perform research on their ancestral lands. Special thanks to Jonathan Scordino and Elizabeth Allyn of Makah Fisheries Management for being the driving force behind scat collection during a particularly stormy winter data collection season. Thanks to my contributors from Washington Department of Fish and Wildlife, Monique Lance, Sarah Brown, and William W. Walker, whose expert analysis was critical to this project. Thanks to Dyanna Lambourn at the Marine Mammal Stranding Network, Dr. David Rosen and the Vancouver Aquarium, the Marine Mammal Center, and Nathan Guilford for obtaining known sex scat samples.

The support of my entire committee has pushed me to become a thoughtful, well-rounded scientist. To Dr. Alejandro Acevedo-Gutiérrez, your unwavering support, reliability and willingness to edit the same bodies of work repeatedly was critical to me finishing this project. To Dr. Dietmar Schwarz, thank you for letting me take over your lab and your email inbox. Thank you for being my sounding board, sharing statistics musings, comparing weekend adventure photos and letting me borrow your dog. A very special thanks to Adrianne Akmajian, who not only obtained funding for this project, but has been a constant source of advice, collaboration and guidance. Thank you for the countless hours of phone calls, mostly centered around Steller sea lions, but with a plethora of valuable tangents.

The COVID-19 pandemic encouraged me to lean on the support of the scientific community and continue research despite shutdowns. To Madison Gard and Holland Conwell, thank you for your patience and laughter as we navigated scat cleaning together. Madison, thank you for going beyond the funding of the project to develop your own independent research and to take some lab work off my hands. To Peter Thut, thank you for finding me a designated scat space, and for being the only friendly face I saw in the WWU Biology building for a long time. To Dr. Kathryn Sobocinksi and Dr. Rebecca Bunn, your coursework and mentorship was critical in developing the statistical analysis and fisheries perspectives within the project. To Dr. Benjamin Nelson, Dr. Austen Thomas, and Madelyn Volker thank you for offering your expertise on these data and for being constantly available to answer my long lists of questions.

Of course, to my friends and family who have supported me through two years of graduate school. To my mom, your strength, resilience and compassion has been a constant source of inspiration. Thank you for dropping everything to drive up to Bellingham with some matzo ball soup and a hug on multiple occasions in the past two years to support me. To my brother, Ian Lewis, thank you for the pet decompression time, climbing sessions, and constant companionship. Although we sometimes miss each other's phone calls, I know I can count on you to pick up if I call 15 times in a row, in the case that one of us gets injured. To my grandparents, I'm extremely lucky to have you support me and encourage me to be a scientist. To Cannon Mallory, thank you for dropping everything to help develop a qPCR assay, perform last minute edits (while I was actively working on the document), drive me to the lab to process scats while still on crutches, and for always taking me to the woods when I needed to recenter. To Kathleen McKeegan, thank you for being my sanity check, my statistics consult, my live-in editor, best friend and most importantly, my roommate. Finally, to my dad who never got to see this adventure. Thank you for inspiring me to look closer and ask questions from a young age. I'm honored to be pursuing a career in Marine Biology like you always wished you could.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

GENERAL BACKGROUND

Despite decreasing numbers taken by commercial and recreational fishing, Chinook salmon (*Oncorhynchus tshawytscha*) populations originating from estuaries within the Salish Sea and from rivers systems along the Pacific coast have continued to decline (Riddell et al. 2018). These populations utilize the Strait of Juan de Fuca and the northwest coastal Washington region at various life stages depending on season (Bi et al. 2008, Weitkamp 2010, Riddell et al. 2018). The decline of Chinook salmon has been linked specifically to lower rates of marine survival due to natural mortality, however the mechanisms of this mortality are unknown (Beamish and Mahnken 2001, Riddell et al. 2018). Previous research has estimated that pinnipeds (seals and sea lions) within the Salish Sea are consuming large amounts of Chinook salmon (Adams et al. 2016, Chasco et al. 2017a). However, these estimates are heavily based on the predation impacts near estuarine environments located within the Salish Sea (Lance et al. 2012, Luxa and Acevedo-Gutiérrez 2013, Thomas et al. 2017, 2022). Although diet data for Steller sea lions (*Eumetopias jubatus*) has been previously collected along the northwest coast of Washington in 2013, increasing abundance of Steller sea lions and temporal changes in diet suggest that impacts of predation on prey has changed (Scordino 2010, Scordino et al. 2022a). Consequently, there are spatial and temporal gaps in Steller sea lion diet data that lead to an incomplete understanding of Chinook salmon mortality along the coast of Washington. Driven by these gaps in diet data, this study aimed to update and broaden the Steller sea lion diet dataset along coastal Washington. Further, this study is the first along the coast to use molecular techniques to characterize diet and determine sex of Steller sea lion predators using scat. Together, these approaches are necessary to understand the impacts of Steller sea lions on prey species of conservation concern, such as Chinook salmon.

In this study, I utilized 274 Steller sea lion scats collected between December 2020-August 2021 along the northern Washington coast to assess Steller sea lion diet and potential impacts on Chinook salmon recovery. I profiled the diet of Steller sea lions along coastal Washington using molecular scatology and hard parts analysis to describe the proportions of prey items consumed. Further, I used qPCR methods to determine the sex of the scat depositor and investigate differential diets of Steller sea lions by sex. These updated diet proportions are critical to improve the accuracy of future studies that aim to estimate the biomass of Chinook salmon consumed by Steller sea lions.

In this general introduction, I set the framework for understanding potential impacts of Steller sea lion predation on Chinook salmon on the northern coast of Washington. First, I investigate the historic trends of Steller sea lion populations along the northwest Washington coast. I then outline the importance of indigenous and ecosystem-based frameworks for fisheries management and describe the life history, physiological, and predator-prey dynamics of Steller sea lions and Chinook salmon. Finally, I explore the methods of diet study analysis and biomass calculations that can be used in modeling and ecosystem-based management frameworks.

Study area: current, historical, and cultural use

Study area

The study area is located at the intersection of the Strait of Juan de Fuca and the Pacific Ocean. The surrounding waterways mark the confluence of the northern portion of the California Current and the Strait of Juan de Fuca, forming the Juan de Fuca eddy at the outlet of the Salish Sea estuary system (MacFadyen et al. 2005, Davis et al. 2014). The bathymetry of this region is

characterized by deep canyons surrounded by shallow banks, causing deep water upwelling in the eddy (MacFadyen et al. 2005). The combination of coastal upwelling combined with estuarine mixing from both the Salish Sea and the Columbia River make this the most biologically productive region of the California Current system (MacFadyen et al. 2005, Davis et al. 2014). The Juan de Fuca eddy develops seasonally, resulting in increased primary productivity during the summer, which results in higher trophic level biomass for this region (MacFadyen et al. 2005). This increased biomass influences population dynamics of plankton, in turn impacting forage fish, seabird and marine mammal species in the area (MacFadyen et al. 2005, Davis et al. 2014, Scordino et al. 2022a).

Located on the tip of the Olympic Peninsula in northwest Washington State, the Makah Tribe has utilized these productive waters for marine resource extraction since time immemorial (Wessen and Huelsbeck 2015). When the Tribe signed the Treaty of Neah Bay, they ceded a large portion of their traditional lands to the US government. The Makah Tribe maintained access to their Usual and Accustomed (U&A) fishing grounds, which includes approximately 1,550 square miles of marine waters surrounding the Juan de Fuca eddy ("Treaty of Neah Bay" 1855, Makah Tribe 2020). Although a highly productive area, worldwide declines of fisheries stocks have led the Tribe to enact a rigorous and cautious approach to ecosystem-based management in order to continue to preserve, protect, and sustainably fish out of these waters (Makah Tribe 2020).

Historical pinniped harvest in the region

Historical accounts describe healthy populations of marine mammal species along the Washington coast with significant mortality pressure due to human predation (Etnier 2002, Braje

and Rick 2011). Pre-colonization, coastal tribes along the Olympic Peninsula hunted marine mammals for subsistence harvests of oil, meat, bones, and skins (Etnier 2002, Wray 2002, Sepez 2008, Coté 2010). The Makah Tribe is known for hunting gray whales (*Eschrichtius robustus*) and humpback whales (*Megaptera novaeangliae*). However, the Makah also harvested five pinniped species: primarily northern fur seals (*Callorhinus ursinus*), harbor seals (*Phoca vitulina*), and occasionally Guadalupe fur seals (*Arctocephalus townsendi*), California sea lions (*Zalophus californianus*), and Steller sea lions (Etnier 2002, 2007, Etnier and Sepez 2008, Braje and Rick 2011, Cammen et al. 2019). Of these species, there is evidence that harbor seals, northern fur seals and Steller sea lions had established breeding grounds, known as rookeries, in the area (Etnier 2002, 2007, Braje and Rick 2011, Moss and Losey 2011). Archeological evidence of Steller sea lion take by the Makah Tribe shows very low rates of hunting, likely influenced by the historically low populations (Etnier 2007).

Colonization by European settlers shifted pinniped abundance and distribution due to increased commercial exploitation, as well as culling programs, throughout the Pacific Northwest and specifically within the Makah U&A fishing grounds (Etnier 2007, Braje and Rick 2011). Commercial exploitation of pinniped populations in the 18th century for fur and blubber impacted all six of the pinniped populations in the region (Scheffer 1928, Braje and Rick 2011). Culling programs also impacted pinniped abundance in coastal Washington and the Salish Sea. An estimated 17,133 harbor seals were killed in the state of Washington between 1943 and 1960 due to bounty programs (Newby 1973, Jeffries et al. 2003). Steller sea lions in the region also faced culling in British Columbia, Canada (Bigg 1985). Following the Marine Mammal Protection Act of 1972, these commercial exploitation and culling programs ended, resulting in an increase in harbor seal and Steller sea lion populations along the Washington coast (Bigg

1985). In comparison, Guadalupe fur seal and the northern fur seal populations did not rebound from overextraction, and population estimates remain low in this region (Etnier 2002, Braje and Rick 2011, D'Agnese et al. 2020, Caretta et al. 2020).

Today, harbor seals, Steller sea lions, California sea lions and, occasionally, northern elephant seals (*Mirounga angustirostris*), northern fur seals, and Guadalupe fur seals utilize the Washington coast (D'Agnese et al. 2020, Oleson et al. 2009, Scordino 2010). The most recent stock estimate describes the Washington coast harbor seal population to be stable (Jeffries et al. 2003, Carretta et al. 2013), whereas Steller sea lions and California sea lions have expanded their range and shown increases in population along coastal Washington and British Columbia (Carretta et al. 2013, Trites and Rosen 2019, Allyn and Scordino 2020). In locations where Steller sea lions overlap in range and habitat with northern fur seals and/or California sea lions, these species compete for prey resources as well as haulout availability (Waite et al. 2011, McCue et al. 2021, Scordino et al. 2022a). Fluctuations in populations of harbor seals, Steller sea lions, California sea lions, and northern fur seals along the northwest Washington coast, combined with variation in diet preferences suggest that predation pressure on prey species may change over time (Waite et al. 2012, Chasco et al. 2017b).

Salmon use and fisheries in the region

Native tribes and First Nations in the Salish Sea basin and coastal Washington have relied on fishery harvest of salmonids, shellfish species and other marine resources since time immemorial and have played a crucial role in the management of this ecosystem (Cederholm et al. 2000, Braje and Rick 2011, Atlas et al. 2021). There is no evidence that subsistence extraction of Chinook salmon has been depleted in response to indigenous use (Butler and Campbell 2004).

In fact, these productive marine regions have been successfully managed by indigenous communities using traditional ecological knowledge (Butler and Campbell 2004, Atlas et al. 2021). Fisheries stocks have responded positively to adaptive management strategies, such as selective fishing technologies and seasonal harvest practices enacted by indigenous resource management in the United States and Canada (Jones et al. 2010, Petersen et al. 2020, Atlas et al. 2021).

Today, the tribes located in the Salish Sea Basin or along the coast of Pacific Ocean within Washington are co-managers of the fisheries stocks within the state (Makah Tribe 2020). Among them, the Makah Tribe has a strong cultural history of marine resource extraction (Coté 2010, Braje and Rick 2011). Historically, the Makah relied on marine mammals, fish, shellfish, kelp and marine invertebrates as staples in their diet (Swan 1870, Sepez 2008). A treaty with the United States secured these subsistence and traditional food harvesting within the context of the colonial government ("Treaty of Neah Bay" 1855). Today the Makah Tribe manages both tribal and recreational fishing of salmonids in their U&A fishing grounds. Further, the Makah Tribe has treaty rights to the harvest of all fish, shellfish, and marine mammals within its U&A, specific examples include Pacific hake (*Merluccius productus*), Pacific halibut (*Hippoglossus stenolepis*), and rockfish (*Sebastes* spp.) (Makah Tribe 2020, Johnson et al. 2021). Thus, the Tribe has a vested interest in protecting the natural marine resources in northwest Washington and uses ecosystem-based management combined with traditional ecological knowledge to best inform management practices in the region.

Species of interest: Steller sea lions and Chinook salmon

Steller sea lion life history, behavior, and food habits

Steller sea lions are the largest species of pinniped within the Otariidae or "eared seals" family. Steller sea lions establish rookeries on land and disperse during non-breeding months (Womble et al. 2009), resulting in alternative haulout sites and vacant rookeries in winter months and changing ratios of male to female individuals at haulout sites (Braje and Rick 2011). The distribution of the eastern distinct population segment (EDPS) of Steller sea lions spans from California to southeast Alaska (Carretta et al. 2013). The EDPS of Steller sea lions on average has increased around 3.22-4.25% yearly from 1986 to 2017, and is not currently listed as an endangered population (Muto et al. 2020). This increase is characterized by drastic shifts in distribution, where Steller sea lion numbers are increasing and expanding at the northern end of their range, but decreasing in areas closer to California (Pitcher et al. 2007). Along the Makah U&A fishing grounds, Steller sea lion counts at haulouts have increased at 7.9% a year between 2010 and 2018 (Allyn and Scordino 2020). Although this region has historically been utilized by Steller sea lions for non-breeding haulouts year round (Akmajian et al. 2017), the first known recorded rookery was established within the past 10 years, which may contribute to the rapid growth of this local group in comparison with the overall growth rate of the EDPS (Carretta et al. 2018, Allyn and Scordino 2020, Scordino et al. 2022a).

Steller sea lions, like many other pinniped species, are generalist predators with a wide variety of prey preferences that often reflect prey availability (Bredesen et al. 2006). Trends in Steller sea lion diet preferences in the wild vary depending on location and season (Kastelein et al. 1990, Bredesen et al. 2006, Trites and Calkins 2008). Studies of Steller sea lions in Southeast

Alaska show drastic seasonal variation in haulout site use and suggest that their use is correlated with foraging strategy based on the availability of prey (Bredesen et al. 2006, Womble et al. 2009). Diets of Steller sea lions within the EDPS vary in top prey items recovered by hard parts analysis across location. Top prey species (>5% split sample frequency of occurrence) found in Steller sea lion diets in southeast Alaska from 2001-2004 include walleye pollock (*Theragra chalcogramma*), Pacific herring (*Clupea pallasii*), Pacific hake, and arrowtooth flounder (*Atheresthes stomias*) (Tollit et al. 2015). In contrast, top prey items (>20% frequency of occurrence) recovered from Steller sea lion diet in coastal Oregon and northern California between 1987-2007 were Pacific hake, salmonids, skates (*Rajidae* spp.) and Pacific lamprey (*Lampetra tridentata*) (Riemer et al. 2011). In northwest Washington, top prey families (>5% split sample frequency of occurrence) recovered from Steller sea lion scats collected between 2010-2013 were clupeids, rockfish, skates, salmonids, flatfishes, dogfishes, and Pacific hake (Scordino et al. 2022a). It is also critical to note that many of the top prey items such as Pacific herring, walleye pollock, Pacific hake, and salmonids are also important species for fisheries extraction in the Pacific Northwest.

The biomass of salmon consumed by Steller sea lions along the northwest coast of Washington was approximately 1330 metric tons of salmonids per year from 2010-2013 (Scordino et al. 2022a). Since these data were collected, the abundance of coastal Washington Steller sea lions have increased (Allyn and Scordino 2020). Hence, updated diet data are necessary to determine more recent impact of predation on salmonids. Scordino et al. (2022a) investigated only the recovered hard parts from scats, which do not allow for species level identification of salmonids present in diet. Genetic analysis of salmonid hard remains found in these scats assumed that all hard remains identified to salmonids within a scat were

representative of one prey item or species, and thus may not accurately capture the relative proportion of salmonid species consumed, as individuals may be consuming multiple salmon individuals of different species (Scordino et al. 2022b). Recent developments in molecular identification (Thomas et al. 2014) allow for higher taxonomic resolution and thus, the data collected for this thesis could provide insight into the proportion and biomass of Chinook salmon consumed.

Seasonality, sex, and reproductive status collectively influence the foraging habits of Steller sea lions. Steller sea lions are sexually dimorphic, male Steller sea lions are on average 2.5 times heavier and require approximately twice as much prey (by weight in kg) than females in both captive and wild settings (Kastelein et al. 1990, Winship et al. 2001, Trites and Calkins 2008). Reproductive females remain closer to rookery sites and require higher food intake while actively pregnant or lactating, peaking in caloric demands during the spring breeding and pupping season (Kastelein et al. 1990, Olivier et al. 2022). Spring also correlates with increases in male prey consumption as they prepare for fasting during the breeding season (Womble et al. 2005). Therefore, the sex of the predator can influence diet preferences, prey amounts, and foraging strategy, due to these temporal and spatial variations (Trites and Calkins 2008).

Differences in nutritional demands based on sex and seasonality also influence prey preference of individual Steller sea lions (Kastelein et al. 1990, Womble et al. 2005, Trites and Calkins 2008). Case studies of captive sea lions and foraging pattern studies of wild populations in Alaska confirm sex-biased prey preference of Steller sea lions (Kastelein et al. 1990, Merrick and Loughlin 1997, Merrick et al. 1997). Sex-specific specialization of prey choice by other pinniped species, specifically harbor seals, show differential consumption of prey items, specifically salmonids (Schwarz et al. 2018). Thus, the pressure of predation on prey items

depends on seasonality and demographics of the predator stock (Kastelein et al. 1990). This thesis aims to document the impacts of sex-biased predation of Steller sea lions using novel molecular techniques.

Chinook salmon life history

Chinook salmon spawn in river systems and migrate towards the ocean (Healey 1991). The coast of Washington and the intersection of the Pacific Ocean and the Strait of Juan de Fuca are utilized by a variety of Chinook salmon stocks all with differing life histories and migratory trends correlated with the timing of their return to natal spawning grounds (Healey 1991, Shelton et al. 2019). These stocks include Chinook salmon originating from natal rivers and streams throughout the eastern Pacific coastline, with salmon migrating from the California, Oregon, Washington and British Columbia coasts, as well as from within the Salish Sea, and along Vancouver Island (Weitkamp 2010, Riddell et al. 2018). The primary natal regions for Chinook salmon utilizing the marine areas of northwest Washington are streams and rivers in the Salish Sea, the Columbia River, and coastal Washington (Riddell et al. 2018).

The density, size, age class and ocean residency of Chinook salmon is specific to river-of-origin and run timing (Weitkamp 2010, Riddell et al. 2018, Losee et al. 2019, Shelton et al. 2019). Thus, the population structure of Chinook salmon off the northwest Washington coast depends on season. Juvenile salmon from these multiple subpopulations aggregate in high concentrations along the outer coast of Washington during the late fall and winter (Pearcy and Fisher 1990, Bi et al. 2008). The transition from juvenile to adult Chinook salmon occurs during their ocean residency, which varies in duration from 2-4 years (Riddell et al. 2018). The location and duration of ocean residency varies depending on subpopulation; some stocks remain close to

their natal streams and coastlines, while others migrate northward towards Alaska (Bi et al. 2008). To complete their lifecycle, adult spawners must then return to their natal streams and reverse their migratory path, resulting in higher aggregations of adult Chinook passing through the northwest Washington coast during the spring and summer (Bi et al. 2008, Trudel et al. 2009, Weitkamp 2010).

Despite decreases in fishing efforts, increases in hatchery and recovery efforts, and habitat restoration, Chinook salmon return numbers have been decreasing, indicating that fishery mortality is not the only driver of declines (Nelson et al. 2020, Manishin et al. 2021). Recent hypotheses indicate that increased natural mortality of ocean-phase Chinook salmon, both out-migrating juveniles and later-stage adults, may be contributing to the decline of run returns (Chittenden et al. 2018, Manishin et al. 2021). Hypothesis-driven statistical models of Chinook marine survival indicate that predation, hatchery timing and anthropogenic impacts such as habitat degradation may be the strongest predictors of decline (Sobocinski et al. 2021). Along the Washington coast, coded wire tag recovery of hatchery Chinook salmon confirms that juvenile survivability is decreasing (Shelton et al. 2019). Juvenile fish mortality can drive decreases in recruitment, as explained by the "critical period" hypothesis, which states that early mortality is a driver of population decline (Hjort 1914, Beamish and Mahnken 2001, Riddell et al. 2018).

Natural mortality of juvenile Chinook salmon is influenced by a variety of oceanographic factors, such as changes in coastal upwelling, and biological controls, such as predation (Bi et al. 2008). As the ocean climate warms and strong upwelling events become less likely and less frequent, the distribution of juvenile salmonids may change along the Washington coast (Cederholm et al. 2000). Although the quantity of available marine habitat is unchanged, weaker upwelling trends have correlated to shifts in distribution of juvenile Coho salmon (*O. kisutch*)

towards the coast and these shifts may indicate changes in juvenile Chinook distribution (Fisher and Pearcy 1988, Bi et al. 2008). These distribution shifts increase vulnerability to predation by opportunistic predators, such as marine mammals, marine birds, and other fishes, as opportunistic predation reflects prey availability in an area (Fisher and Pearcy 1988, Cederholm et al. 2000, Luxa and Acevedo-Gutiérrez 2013). Other biological vulnerabilities of early marine phase Chinook salmon include decreases in the availability of prey items such as zooplankton and forage fish, such as Pacific Herring (Bi et al. 2008, Chittenden et al. 2018).

Investigating the trends of predator consumption of Chinook salmon may help understand rates of natural mortality. A meta-analysis of marine mammal diet studies spanning from Alaska to California, show variation in the frequency of salmonids in pinniped diet depending on season and species (Adams et al. 2016). As generalist predators with a broad variability in diet, pinnipeds may benefit and disproportionately impact prey species that undergo seasonal migrations, including salmonids (Womble et al. 2005, 2009, Thomas et al. 2011). Steller sea lion predation has been implicated in the decline of the Fraser Rivers sockeye salmon (*O. nerka*) run due to the overlap of sea lion haulouts and sockeye migration (Walters et al. 2020). Further, predictability of prey presence due to seasonal migration increases probability of Steller sea lion predation (Tollit et al. 2015, Fritz et al. 2019), indicating that Chinook salmon may be disproportionately vulnerable to sea lion foraging along their migration path. Determining the rate of predation where there is spatial overlap between prey migration and Steller sea lion foraging is necessary for quantifying natural mortality. Further, bioenergetics modeling has shown that low proportions of Chinook salmon in marine mammal diet may still result in high consumption of Chinook salmon biomass (Chasco et al. 2017a, 2017b, Nelson et al. 2021).

Pinniped diet study methodologies

Hard part diet studies

Hard parts analysis of scat involves the identification of all structures remaining in fecal material after cleaning, such as boney pieces (i.e. otoliths) and cephalopod beaks (Bowen and Iverson 2013). Hard parts studies have been the standard for identifying prey items in fecal samples, stomach contents and regurgitation (Bowen and Iverson 2013). The majority of previous diet studies on Steller sea lions in the EDPS have focused on hard part identification from scat samples collected (Bredesen et al. 2006, Trites and Calkins 2008, Tollit et al. 2015, Akmajian et al. 2017, Scordino et al. 2022a). Although informative, hard part analysis alone may not provide an accurate picture of diet proportions due to sampling and analysis bias (Lance et al. 2001, Cottrell and Trites 2002, Tollit et al. 2009, Bowen and Iverson 2013, Tollit et al. 2015).

Error and biases in hard parts diet studies include collection challenges, partial prey consumption, disproportionate degradation of prey items, and lack of species-level identification (Tollit et al. 2010, Bowen and Iverson 2013). Recovery of hard parts from scats may not be possible when scats are collected from haulout sites where tides quickly weather or wash away portions of the sample, and recovery of consistent scat samples over seasons may be challenging (Akmajian et al. 2017). These studies are inherently biased towards prey structures that are more easily recovered after digestion, such as larger boney structures (Tollit et al. 2009, 2015). Certain prey items may have fewer or no boney structures (i.e. cephalopods) or hard parts may be disproportionately damaged through the digestion process (Merrick and Loughlin 1997, Cottrell and Trites 2002, Tollit et al. 2015).

Feeding behavior of the predator may bias recovery of hard parts as well (Tollit et al. 2010, Bowen and Iverson 2013). Regurgitation, or "spews" of larger hard parts by Steller sea lions may bias hard parts analysis towards the recovery of smaller hard parts found in scat (Gudmundson and Ream 2000, Waite et al. 2011). Studies of sea lion diets have noted specific biases of salmon otolith recovery due to foraging habits (Cottrell and Trites 2002, Sweeney and Harvey 2011). For example, Steller sea lions tend to bite the head of larger salmon prior to consumption, creating a reduction in larger prey item recovery as this behavior may increase the likelihood of otolith damage (Cottrell and Trites 2002). Diet studies analyzing Steller sea lion consumption of salmonids must acknowledge the bias towards smaller individuals and underestimation of larger salmonids (Bowen and Iverson 2013).

Species-level identification of salmonid hard parts is only possible in scats where otoliths are present (Tollit et al. 2010, Bowen and Iverson 2013). Salmon otoliths degrade within the digestive tract, decreasing the quantity and quality of otoliths recovered (Lance et al. 2001, Bowen and Iverson 2013, Tollit et al. 2015). In captive Steller sea lion scats, only 10% of salmonid otoliths were recovered, indicating underestimation of salmon in previous hard part studies that relied exclusively on otoliths for identification of prey species (Cottrell and Trites 2002). These captive diet studies have developed correction factors for certain prey species to accommodate for differential digestion (Cottrell and Trites 2002). Although correction factors may be used, these cannot fully account for the lack of otolith recovery or complete absence of hard parts from a prey item (Cottrell and Trites 2002). The prey identifications obtained from hard parts analysis are reported to the lowest taxonomic level identified, which often results in family level identification of families where speciation is difficult, such as salmonids and rockfishes (Akmajian et al. 2017, Scordino et al. 2022a). Therefore, diet proportions determined

via hard parts analyses may provide less species specific information than those obtained with new molecular methodologies (Tollit et al. 2015, Thomas et al. 2016).

Methods used to quantify prey taxa from hard parts remains are most frequently two statistics: frequency of occurrence (described in Tirasin & Jørgensen, 1999) and split-sample frequency of occurrence (Olesiuk et al. 1990). Frequency of occurrence (FO) is a metric of prey frequency based on the presence or absence of a prey taxa within a collection of scat samples (Tollit et al. 2009). FO is calculated as a percentage by diving the number of scats containing a prey item by the total number of scats (equation 1).

Equation 1: Frequency of occurrence (FO_i)

$$FO_i = \frac{\sum_{k=1}^{s} O_{ik}}{s}$$

where;

O_i = 0 if taxon i is absent in fecal k

= 1 if taxon i is present in fecal k

s = total number of fecal samples that contained prey

Unfortunately, FO does not account for the relative proportion of prey remains extracted from each scat and cannot be used to estimate relative proportions of prey within a scat sample. However, this summary statistic is likely least impacted by differential hard parts recovery and can provide a quantitative measure of diet trends in a population (Riemer et al. 2011).

Split sample frequency of occurrence (SSFO) also relies on the presence/absence of prey in a scat sample, but it compares the relative proportion of prey item within each sample and across a population (Olesiuk et al. 1990). SSFO is also expressed as a percentage, so that each

prey item in each scat sample is equal and then averaged across all scats for each prey type. If there are 4 prey taxa present in one sample, each prey taxa are represented as 25%, then this percentage is averaged across all scats for each taxa (equation 2).

Equation 2: Split sample frequency of occurrence ($SSFO_i$)

$$SSFO_i = \frac{\sum_{k=1}^{s} O_{ik}/O_i}{s}$$

where;

O_{ik} = 0 if taxon i is absent in fecal k

= 1 if taxon i is present in fecal k

O_k = total number of all taxa present in fecal k

s = total number of fecal samples that contained prey

Conversely to FO, SSFO overestimates the presence of small prey and underestimates the presence of large prey, as it is assumed that all prey items are consumed at the same proportion (Olesiuk et al. 1990). Despite fundamental issues with hard parts recovery in scat collection, hard parts identification with cautious analysis can provide invaluable information regarding prey presence and age of prey using otolith measurements (Bowen and Iverson 2013, Thomas et al. 2017).

Molecular scatology

In the past 20 years, molecular techniques for pinniped scat analysis to obtain diet data has undergone rapid development, reflecting the increased accessibility and decreased cost of molecular methods. Polymerase chain reaction (PCR) technology was first used with pinniped

scat to investigate species and sex of the seal depositor (Reed et al. 1997). PCR amplifies sections of DNA using primers specific to target sequences which allows the researcher to determine presence of targeted DNA. Prior to diet analysis in pinnipeds, PCR detection of prey remains in scat were performed on a variety of mammalian predators (Höss et al. 1992, Jarman et al. 2004). Initial molecular investigation of pinniped diet used salmonid-specific PCR to amplify mitochondrial DNA (mtDNA) in salmon bones to determine the species of salmon found in harbor seal fecal samples (Orr et al. 2004, Purcell et al. 2004, Kvitrud et al. 2005).

Starting in 2005, Deagle and Tollit validated novel molecular methods to analyze captive Steller sea lion scats with known prey concentrations (Deagle et al. 2005, Deagle and Tollit 2007, Tollit et al. 2009). Their first study developed a protocol to extract DNA from scat and use PCR methods to interrogate samples for the 16s mtDNA sequences of prey groups (Deagle et al. 2005). PCR products were then used to determine relative proportions of prey items using denaturing gradient gel electrophoresis (DGGE). Shortly after, the same scats were analyzed with multiple qPCR reactions using primers specific to the 16s mtDNA for each prey species (Deagle and Tollit 2007). Finally, Tollit et al. (2009) investigated the use of universal primers (also at the 16s mtDNA region) to amplify genetic sequences from all chordates and all cephalopod prey items and determined prey proportions using DGGE. Estimates from these data showed that 22% more species were identified by DNA analysis in comparison to hard parts identification (Tollit et al. 2009). Further, these results demonstrated that DNA analysis may be used to distinguish between salmonid species when samples lack or contain damaged otoliths (Deagle and Tollit 2007). Although these studies showed promising use of qPCR to determine target prey species within scat, these studies did not allow for the identification of non-target

species. This research also determined that prey mtDNA degraded within fecal material at differential rates, potentially leading to biases in proportion analysis (Deagle and Tollit 2007).

DNA metabarcoding is a protocol that combines the use of universal qPCR primers with next generation sequencing (NGS) to genotype and identify species within samples simultaneously. Using the universal qPCR primers removes the need for species specific assays which allows researchers to investigate the entire breadth of species DNA found in environmental DNA samples (Hebert et al. 2003). Building on their previous research, Deagle et al. (2013) validated the method of DNA metabarcoding to determine proportions of prey items in captive harbor seal scats. Following the use of molecular methods with captive seal scats, Thomas et al. (2017) implemented a combination of both hard parts analysis and DNA metabarcoding to identify prey items and relative proportions in wild harbor seal scat collected in the Salish Sea. This study demonstrated that DNA metabarcoding with universal primers at the 16s mtDNA region for chordate and cephalopods could identify all potential prey items and relative prey proportions (Thomas et al. 2017). Given the increased resolution of these methods, DNA metabarcoding methods have also been used in other pinniped species, including Australian sea lions (*Neophoca cinerea*) and northern fur seals (Berry et al. 2017, Hardy et al. 2017, Jeanniard-Du-Dot et al. 2017).

Although increased species resolution is critical in determining diet diversity of pinniped predators, digestion and amplification biases can occur, influencing the relative read proportion used to reconstruct diet via DNA metabarcoding (Thomas et al. 2014, 2016). In an attempt to investigate and correct for differential digestion of prey species, studies worked to determine species specific tissue correction factors and relative correction factors to reduce digestion bias in samples extracted from harbor seals (Thomas et al. 2014, 2016). Although helpful in obtaining

the actual biomass consumed from prey, these studies are time intensive, and must be able to identify all potential prey items. It is suggested that using relative read abundance (quantity of DNA reads) from a certain prey item may be used to estimate biomass consumed, with appropriate correction factors and acknowledgement of bias (Thomas et al. 2014, 2016). Subsequent research suggests that despite sources of potential error, relative abundance of prey items from DNA metabarcoding is more reflective of diet than occurrence based (presence/absence) DNA metabarcoding data (Deagle et al. 2019) and is preferred in comparison to the split sample frequency of occurrence (SSFO) in hard parts analysis for studies with the aim of biomass reconstruction and ecosystem modeling (Deagle et al. 2019, Thomas et al. 2022).

To further understand diet of pinniped predators using scat, qPCR methods have also been developed to identify sex of the scat depositor, which may help inform demographics of sampled populations. Schwarz et al. (2018) adapted the qPCR analysis method developed by Matejusová et al. (2013) to determine the sex of harbor seals from scat and compare their diet across spatial and temporal scales in the Salish Sea. These molecular methods have been implemented to compare individual harbor seals prey specialization based on sex, showing that male harbor seals preferentially feed on Chinook salmon (Voelker et al. 2020). This methodology has not been used for Steller sea lion or other sea lion scat studies, and adapting this method for sea lion species would allow further exploration of diet preferences of male and female Steller sea lions in wild populations.

Currently, there are no methodologies to determine relative age of prey items via molecular analysis. Pairing metabarcoding data with hard part data collection is critical to estimate impacts of predation on differing life stages of prey items (Thomas et al. 2017). To my knowledge, this study will be the first to combine qPCR predator sexing, DNA metabarcoding

species identification, and hard part identification to describe Steller sea lion diet. The combination of these methods leads to a robust profile of diet as well as diet proportions that may be used for calculating prey biomass consumed by Steller sea lions along the coast of northwest Washington.

Estimating predator consumption of prey biomass

Investigating pinniped influence on the declines of fisheries stocks has become necessary (Laake et al. 2002). Increases in frequency of occurrence of salmon in Steller sea lion diets indicates increasing impacts of predation pressure on Chinook salmon populations (Adams et al. 2016). However, diet data alone cannot be extrapolated to the biomass of prey population consumed by a predator (Thomas et al. 2016, Deagle et al. 2019). Hence, biomass estimation models are necessary for quantifying the impact of Steller sea lion predation on Chinook salmon (Adams et al. 2016) and can be incorporated into existing models to better inform management of predator prey interactions (Laake et al. 2002, Walters et al. 2020).

Multiple prey biomass and bioenergetics consumption models have been developed to describe marine mammal consumption of Chinook salmon in the Salish Sea (Chasco et al. 2017a) and along the entire west coast (Chasco et al. 2017a, Scordino et al. 2022a). Along the west coast, the estimated biomass of Chinook salmon consumed by Steller sea lions increased from 300 metric tons in 1975 to 1,200 metric tons in 2015 (Chasco et al., 2017a). Pinniped diet proportions used within the Chasco et al (2017a) bioenergetics models are based on DNA metabarcoding studies for harbor seal diets and hard parts analysis of Steller and California sea lions (Scordino et al. 2013, Chasco et al. 2017b).

The use of hard parts only diet data in previous biomass modeling suggests the underestimation of Steller sea lion consumption of Chinook salmon, as previous research shows that salmon is recovered at lower rates in hard parts diet proportions than is consumed by Steller sea lions (Cottrell and Trites 2002). Diet proportion data for Chinook consumption by Steller sea lions along the coat of Washington was collected over ten years ago and at current growth rates (Allyn and Scordino 2020) the abundance of Steller sea lions has likely doubled. This study aims to update diet proportions to better inform the resolution of bioenergetics modeling and further explore the impacts of this Steller sea lion predation on Chinook salmon.

FORAGING ECOLOGY OF SEXUALLY-DIMORPHIC MARINE GENERALIST PREDATORS: DESCRIBING STELLER SEA LION DIET ALONG THE NORTHERN WASHINGTON COAST

INTRODUCTION

Generalist predators consume prey items from a variety of trophic levels and thus, they have a significant impact on prey abundance and diversity (Ellingsen et al. 2020). The effect of generalist predation on prey populations depends on predator size, abundance and diet (Womble et al. 2009, Tollit et al. 2015, 2017, Chasco et al. 2017a, Steingass 2017, Schwarz et al. 2018, Voelker et al. 2020). For generalist predator populations, diet reconstruction is often challenging, as diet can vary significantly depending on prey abundance and availability (Terraube and Arroyo 2011). Further, generalist predators are highly opportunistic and have a wide breadth of prey that vary over time. For example, Eurasian badgers (*Meles meles*) exhibit both seasonal as well as annual shifts in diet preferences between fruits and arthropods (Rosalino et al. 2005). The trends in Eurasian badger diet were not correlated to abundance of food type but rather to seasonal specialization of olive as a primary food source (Rosalino et al. 2005). These data suggest that understanding foraging ecology in combination with resource availability is important for understanding generalist preferences (Rosalino et al. 2005). Due to the temporal variability influenced by both foraging habits and environmental variation, the diets of generalist predators need to be analyzed periodically, especially when investigating impacts of predation with the use of ecosystem modeling (Hayden et al. 2014). Moreover ecosystem models used to understand predator-prey relationships rely on thorough understanding of predator diets, and therefore must account for all factors that may influence variation (Essington 2004, Smout et al. 2010).

Seasonal and annual variation in generalist predator diets is well documented, however many studies examining trophic effects of predation assume diet composition is uniform across predator demographics and does not vary depending on individual (Quevedo et al. 2009). However, it is accepted that generalist predators may exhibit intrapopulation variation, variation between individuals within the same population (Bolnick et al. 2003). Multiple studies demonstrate that sex, size, age, and predator morphology explain variation in diet, which may or may not be due to individual specialization (Martins et al. 2008, Tarroux et al. 2012, Nifong et al. 2015, Schwarz et al. 2018). Individual variation in dietary generalists have been observed in longitudinal studies of African elephants (*Loxodonta africana)*, which show large seasonal and annual variation within individual lifetimes (Codron et al. 2012). In contrast, individuals of another generalist population, the southern elephant seal (*Mirounga leonina*), exploit the same prey species throughout their lifetime (Rita et al. 2017). The impacts of these contrasting individual foraging habits within generalist populations suggest that understanding species-specific specialization and dietary tendencies may be critical to disentangle whether generalist populations diet trends can be represented by large spatiotemporal samples, or whether individual specialists may disproportionally contribute to the decline of a prey species. Thus, individual level specialization must be determined in tandem with diet reconstruction to accurately assess impact on prey species within a local population.

Pinnipeds (seals, sea lions, and walruses) are one group of generalist marine predators who demonstrate intrapopulation variation (Kernaléguen et al. 2016, McHuron et al. 2016, Voelker et al. 2020) and thus impact of pinnipeds on prey must be investigated at a local level. Due to the large abundance of harbor seals along the northwest coast of Washington State and the Salish Sea, a wide body of studies have focused on harbor seal diet in these regions. Harbor

seal consumption of important fishery species, such as Pacific salmon (*Oncorhynchus* spp.), has been documented in studies using scat based dietary analysis (Lance et al. 2012, Howard et al. 2013, Luxa and Acevedo-Gutiérrez 2013, Thomas et al. 2017, 2022, Schwarz et al. 2018). Female harbor seals show higher specialization during the spring and summer, thus influencing trophic dynamics differently than male harbor seals within the Salish Sea (Voelker et al. 2020). Describing intrapopulation feeding diversity of pinnipeds in tandem with regional diet data is necessary to understanding potential impacts of a predator species.

Steller sea lions (*Eumetopias jubatus*) are large, sexually-dimorphic generalist predators whose diets vary depending on sex (Merrick and Loughlin 1997, Womble et al. 2005, Trites and Calkins 2008), location (Bredesen et al. 2006, Trites and Calkins 2008) and season (Womble et al. 2009). Steller sea lion diet variation is partially attributed to prey availability and abundance (Bredesen et al. 2006), however other factors, including individual specialization, may play a role in prey selection. Seasonality, sex, and reproductive status collectively influence the nutritional demands of individual Steller sea lions, and thus may also influence foraging behavior and intrapopulation feeding diversity (Kastelein et al. 1990, Winship et al. 2001, Womble et al. 2009, Olivier et al. 2022). The intrapopulation specialization in Steller sea lions is unknown, and further investigation of individual feeding diversity in these sexually dimorphic predators may help describe foraging habits of Steller sea lions within a region.

Along the northwest coast of Washington State, Steller sea lion abundance has increased as much as 7.9% between 2010 and 2018 (Allyn and Scordino 2020), indicating a potential for increased predation pressure on top prey species. Diet studies conducted on Steller sea lions in this region have been limited to identification of hard parts from scats (Scordino 2010, Scordino et al. 2022a). Steller sea lion scats collected in the spring and summer of 1998 along the

northwest coast of Washington show a high frequency of Pacific hake (*Merluccius productus*), followed by spiny dogfish (*Squalus suckleyi*) and starry flounder (*Platichthys stellatus*) as the most common prey species (Scordino 2010). In a study from 2010-2013, frequent prey items shifted to herrings, shads and sardines (Clupeidae), rockfishes (Sebastidae), skates (Rajidae*)*, flatfishes (Pleuronectiformes), Pacific hake and Pacific salmon (Scordino et al. 2022a). Among these top prey groups, are species of conservation concern such as Pacific herring (*Clupea pallasii*), Chinook salmon (*Oncorhynchus tshawytscha*), rockfishes (Sebastidae) (Palsson et al. 2009, Schweigert et al. 2010, Marshall et al. 2016, Thompson et al. 2017). A better appreciation of Steller sea lion intrapopulation diet diversity and diet composition are critical to understanding the potential impacts of this population on prey species of conservation concern along the coast of Washington State.

In this study, I used Steller sea lion scats collected along the coast of Washington to investigate four hypotheses to further understand the foraging ecology of the species. First, I used molecular techniques to investigate whether there were sex-specific differences in diet composition. Second, I quantified the population diet diversity and relative individual specialization of Steller sea lions, and asked whether sex influences relative individual specialization. Third, I examined whether certain trends in relative individual specialization, a proxy for intrapopulation diet diversity, occurred with certain prey species. Lastly, I investigated whether certain prey species correlate with each other to describe general foraging trends of the Steller sea lion along the northwest coast of Washington.

METHODS

Study site

The study area lies at the confluence of the Salish Sea —the inland waters of the Strait of Georgia, Strait of Juan de Fuca and Puget Sound— and the Pacific Ocean (Figure 1). This region at the northernmost end of the California Current is characterized by wind and topography driven upwelling, resulting in a nutrient dense cold water gyre known as the Juan de Fuca Eddy that develops in the summer (MacFadyen et al. 2005). The combination of coastal upwelling combined with estuarine mixing from both the Salish Sea and the Columbia River make this the most biologically productive region of the California Current system, and home to a large biodiversity of fish species (MacFadyen et al. 2005, Davis et al. 2014). This increased biomass influences population dynamics of plankton, in turn impacting forage fish, seabird, and marine mammal species in the area (MacFadyen et al. 2005, 2008, Davis et al. 2014). Marine mammal species include Steller sea lions, which utilize haulout sites along the northwest Washington coast throughout the year (Allyn and Scordino 2020, Scordino et al. 2022a).

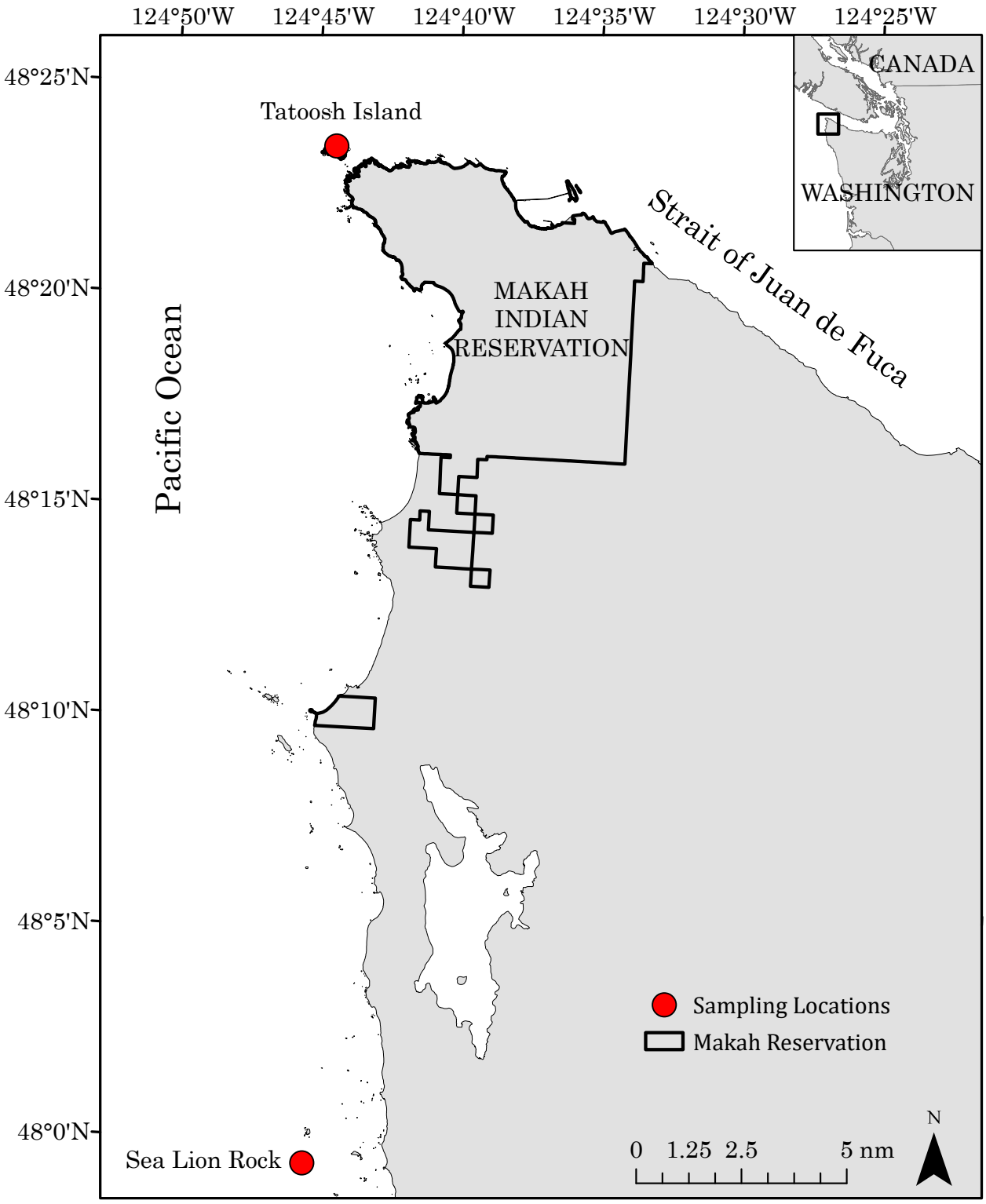

Figure 1. Collection sites of Steller sea lion scats at the confluence of the Pacific Ocean and the Strait of Juan de Fuca, located on the northwest coast of Washington State.

Scat collection and processing

Scats were collected primarily from Tatoosh Island (N 48° 39.463, W 124° 73.809), a year-round haulout complex for Steller sea lions in northwest (Figure 1). At least 90 scats were collected for each season, defined by month as winter: December, January, February; spring: March, April, May; summer: June, July, August. Scat collection was supplemented by collection from a secondary haulout site, Sea Lion Rock (N 47° 99. 224, W 124° 72.653), in months where scat collection was not possible at Tatoosh Island due to absence of Steller sea lions at the haulout, unsafe landing conditions, sea lions hauling out in a location that was inaccessible, or few scats were located on the Tatoosh Island haulout site.

Prior to collection, vessel-based surveys were conducted to count the number of Steller sea lions hauled out at the site, as well as demographic counts by sex and age. Following the methods used in Scordino et al. (2022a), individuals were classified as adult females by size, shape, longer whiskers and presence of dependent pup or juvenile following the methods used in Scordino et al. (2022a). Adult males were identified by larger body size, fore flippers and heads, as well as coarse neck and chest fur. Juveniles were identified as individuals between 1 and roughly 5 years old without the developed sexually dimorphic characteristics. Unweathered, fresh scats were targeted to maximize the quality of DNA recovery, following methodologies previously described for DNA metabarcoding of harbor seal scats (Thomas et al. 2017, Voelker et al. 2020). Briefly, scats were scooped into either Histoplex containers lined with paint strainer bags or Whirl-Pak bags and were frozen until further processing. Frozen scats were then thawed in ethanol and manually homogenized within paint strainer bags to separate hard parts from DNA matrix (Thomas et al. 2016, 2017, Voelker et al. 2020). Paint strainer bags were sealed with zip ties, and washed in a commercial washing machine on a gentle setting (Orr et al. 2003).

Hard parts were then removed from paint strainer bags and additional cleaning occurred using nested sieves (0.5 mm, 1mm, and 2 mm). Hard remains were preserved in ~50% isopropyl alcohol in 20 mL scintillation vials. Dried samples were shipped to Washington Department of Fish and Wildlife (WDFW) for identification. Meanwhile, fecal ethanol slurries were sent to the WDFW Genetics laboratory for DNA extraction and DNA metabarcoding analysis.

Sea lion diet via hard parts analysis

Identification of prey remains was performed by Monique Lance at WDFW. Remains identified included bones (such as otoliths, vertebrae, teeth, and gill rakers), cartilaginous structures, cephalopod pens, and cephalopod beaks. Hard parts were identified to the lowest possible taxonomic classification by comparing sample specimen with reference hard parts under a dissection microscope as described in Lance et al. (2001, 2012). Occasionally, prey items were confidently identified to genus, with a strong likelihood of species level identification. Inability to confirm species was due to either a lack of distinguishable species-specific features or a lack of reference specimen. These prey items were reported within prey family groupings separately from prey species with the indicator "cf". When possible, hard remains were grouped by age class into "juvenile" or "adult" categories based on reference specimens and morphological differences. For salmonids, adults are estimated to be from individual fish larger than ~375 mm. Prey remains identified from juvenile salmonid individuals are assumed to be ocean age zero, typically less than ~300 mm (Nelson et al. 2021).

Results from prey hard part identification were used to determine the split sample frequency of occurrence, used as a proxy for diet proportion. Split sample frequency of occurrence (SSFO) was calculated for each prey item identified to compare the relative

proportion of a prey type within each sample across the entire population of scats collected (Olesiuk et al. 1990):

$$SSFO_i = \frac{\sum_{k=1}^{s} O_{ik}/O_i}{s}$$

where;

O_{ik} = 0 if taxon i is absent in fecal k

= 1 if taxon i is present in fecal k

O_k = total number of all taxa present in fecal k

s = total number of fecal samples that contained prey

Sea lion diet via DNA metabarcoding

DNA was extracted from pelleted DNA matrix using the QIAGEN QIAamp Fast DNA Stool Mini Kit and adjusted protocols for pinnipeds (Deagle et al. 2005). DNA metabarcoding was performed by Dr. Sarah Brown at WDFW, following DNA metabarcoding diet analysis protocols (Thomas et al. 2016, 2017, 2022). Two previously determined PCR assays were used to target and amplify prey sequence regions from extracted DNA: mitochondrial 16S rRNA (16S) and Cytochrome Oxidase I (COI) as described in Thomas et al. (2017). Two qPCR assays (Chordate and Cephalopod primer sets) were used to interrogate for the 16s region to determine relative read abundance (RRA) of vertebrate prey (Deagle et al. 2009, Thomas et al. 2016). The salmon specific minibarcode COI region was used to determine the relative proportion of salmonid species, as the 16S region does not accurately differentiate between the five salmon species found within the study region (Thomas et al. 2017). A blocking oligonucleotide that

matches Steller sea lion sequence (GenBank Accession AB300608.1, 34 bp: ATGGAGCTTCAATTAACTTACCCAATCAGAACT-C3) was used to prevent an overabundance of predator reads.

SequalPrep™ Normalization Plate Kit was used as per manufacturer's instructions to normalize and pool amplicons from 96 individually labeled samples. Sequencing libraries were then prepared from the amplicon pools produced from each PCR reaction and labeled using Kapa LTP Library preparation kit to ligate uniquely labeled adapter sequences to each pool. To increase the sequencing depth of the 16s region, concentration of 16s amplicons pooled was 3x the concentration of COI amplicons. These libraries were then further pooled, and the DNA sequenced, which was performed on an Illumina MiSeq using the MiSeq Reagent Kit v3 (300 cycle) for SE 300bp reads.

Bioinformatics processes were performed following methods described in (Thomas et al. 2016) developed for analysis of harbor seal scat in the Salish Sea, with a few notable differences to account for changes in location of study and the analysis of Steller sea lion scats. The reference nucleotide Basic Local Alignment Search Tool (BLAST) database used to assign DNA sequences to prey species was adapted from the database designed by Thomas et al., 2017 with the addition of species previously found in Steller sea lion diets along coastal Washington. Database assembly was refined after DNA was run on sample sequence reads, following reference database assembly described in Thomas et al. 2017. Metagenomics analysis was performed in QIIME 2 to demultiplex and remove adapter and index sequences (Bolyen et al. 2019). MiSeq post processing automatically sorted sequences by amplicon pool using the indexed TruSeqTM adapter sequences. "DADA2" was used to denoise, dereplicate, merge, and filter and truncate sequences (Callahan et al. 2016). Amplicons were truncated at 280 bp, based

on decreases in the quality scores assigned within DADA2. Decontamination was performed for each plate to remove the proportional number of prey sequence reads that occur in negative controls from samples using the R package *microDecon* (McKnight et al. 2019). The R statistical software package "taxonomizr" was used to assign taxonomic identification to all species within the database. Decontaminated sequences were assigned to a species based on the best match in the updated reference database requiring a 99% match between reference sequence and sample sequence (threshold BLASTN e-value < 1e-20 and a minimum identity of 0.99).

The proportions of each prey species were quantified as the number of reads from a prey species divided by the total number of prey reads within an individual sample, to determine the relative read abundance, or RRA (Thomas et al. 2016). Prey items within a scat were excluded from subsequent analysis if they comprised < 1% of identified prey DNA sequences within a sample, to account for eDNA or secondary prey items. To remove samples where DNA recovery was low, I excluded samples with less than 20 prey reads. Previous data sets use threshold of >10 reads per sample, but improvements in sequencing and increased read recovery suggest that increasing this threshold is necessary for removing samples to prevent sampling error bias (Drake et al. 2022). I determined the new threshold of excluding samples with <20 prey reads with binomial sampling error. The probability that removing a sample with this threshold would result in the removal of a prey item within 15% of the actual diet is 0.05. With this low sampling error, I determined that this higher threshold would not result in the removal of important prey items from the overall diet. The total proportion of salmonid within a scat was determined by the proportion of salmonid reads compared to the total reads as determined by the 16S amplification. The proportion of salmon DNA within each sample was then divided among salmon species using the fractions quantified by COI (Thomas et al. 2017).

Sex determination of Steller sea lion scats

Following DNA extraction, I conducted qPCR to determine the sex of the Steller sea lion depositor. I modified protocols employed for harbor seal scat (Matejusová et al. 2013, Rothstein 2015, Schwarz et al. 2018, Voelker et al. 2020). Specifically, I used TaqMan Quantitative polymerase chain reaction (qPCR) combined with novel primer/probe combinations to determine the presence of X- and Y-chromosomally linked markers (ZFX and SRY) within scats. Custom primer and probe pairs were designed using the primer design wizard tool on Benchling (2022, accessible at https://benchling.com/zoeklewis/f_/THZn2gKq-probe-designing/). The qPCR assays targeting X chromosomes were designed using the reference sequence for the Steller sea lion x-linked zinc finger protein (ZFX) region (GenBank Accession number: NW_020998717.1) and sex determining region Y protein (SRY) gene (GeneBank Accession number: AY424649.1). Novel qPCR assays were tested against the entire known Steller sea lion genome to identify possible overlap between qPCR assays and non-target regions using BLAST. ZFX primer and probe sequences showed no overlap with the SRY region, and SRY primer and probe sequences showed no overlap with the ZFX region. Each sample was run four times, 2 replicates with both probes, following the protocols described in Schwarz et al. (2018) and Voelker et al. (2020).

Table 1. Sequences of novel primer and probe Taqman assays for sex determination of Steller sea lions.

Assay	Size of amplicon (bp)	Forward primer (5'-3')	Reverse primer (5'-3')	Probe (5'-3')
Ej ZFX	22	GCCCCAGCCCTCACGTGTTAAC	TAGCCTCGCCACTGGCCTTTCT	CCTCGTCGTTTGTTGTGGGAGTGGC
Ej SRY	22	CCAACTCGCTGCTGCAACAGGA	GTCAGCGGACAGATGCGTAGCC	CTCCGTGGCAGTCCGGAAACCT

Data analysis

Sex ratios as determined by qPCR and observed demographic counts

Demographic counts were conducted for the haulout region from which scats were collected to determine the number of adult females, adult males and juvenile individuals. Observed demographic counts were conducted prior to landing for collection. Pup counts were excluded from sex ratio estimates, as they were nursing during the study period, and thus not accounted for in scat analysis. I used expected sex-proportions of juveniles to estimate the number of female and male juveniles based on observed counts and determine the overall ratio of male to females, regardless of age. The expected sex-proportion of juvenile Steller sea lions was estimated to be 54.4% female and 45.6% male, as estimated by Scordino et al. (2022a) for this region using survival estimates of age 0 to 5 Steller sea lions (Wright et al. 2017). A Pearson's test was run to test for correlations between monthly sex ratios obtained from observed counts and a Shapiro test was conducted to verify normality of qPCR and observed sex ratios prior to the analysis using the R package 'rstatix' version 0.7.0 (Kassambara 2021).

Diet composition in male and female Steller sea lions

I used permutation multivariate analysis of variance (PERMANOVA) to test whether diet composition, determined by relative read abundance of prey species, across individual samples was different among seasons using Bray-Curtis dissimilarity matrix with the "adonis2" function in the R package 'vegan' version 2.6-2 (Oksanen et al. 2022). PERMANOVA was then used to assess whether diet composition was different due to sex within each season. To further explain variation in diet composition, I also ran PERMANOVA to see whether diet composition varied

due to month, sex and season. PERMANOVA is slightly sensitive to unbalanced data sets, but only when data display overdispersion around the group centroid (Anderson and Walsh 2013). Therefore, I analyzed the homogeneity of multivariate dispersion to assess beta diversity within analysis groups using the "betadiper" function with Bray-Curtis dissimilarity measure also in the R package 'vegan' version 2.6.2 (Oksanen et al. 2022). I used a permutation test to compare the group mean dispersions. When significantly over dispersed and the sample sizes were equal, I used Tukey Honest Significant Differences to compare the group mean dispersions and determine the confidence intervals. The strata argument within the "adonis2" was used to constrain permutations within haulout sites to account for variation (Oksanen et al. 2022). All PERMANOVA and permutation-based dispersion tests were calculated with 10,000 replicates.

Diet diversity and short-term relative individual diet specialization

To further investigate diet diversity at a population level, I calculated Shannon diversity indices using the relative read abundance and the lowest taxonomic identification to determine overall niche breadth for samples grouped by season and sex, using the 'Shannon' function in the R package 'vegan' version 2.6-2 (Oksanen et al. 2022). Increased Shannon index values for a group indicate increased diet diversity of the population. The equation used to calculate Shannon diversity index values (H) is as follows:

$$H = -\sum_j (p_j \times \ln(p_j))$$

where;

p_j is the average relative read abundance of species j across all samples

Relative individual diet specialization was investigated using proportional similarity indices and relative read abundance as described in Voelker et al. (2020). Diet reconstruction collected from DNA metabarcoding of scats reflects short term dietary habits, and thus can only indicate specialization within short periods (Voelker et al. 2020). Relative individual level specialization is represented by proportional similarity indices (PS_i) which is defined as (Bolnick et al. 2002):

$$PS_i = 1 - 0.5 \sum_j |p_{ij} - q_j|$$

where;

p_{ij} is proportion of resource *j* used by individual *i*
q_j is the proportion of resource *j* used by the population

Population groups used to calculate PS_i values were informed by PERMANOVA results, as PS_i assumes that all individuals within a population are using the same resource pool (Bolnick et al. 2003). In this analysis, the population used to calculate PS_i was defined as a group of Steller sea lion scats collected in the same month to compare intrapopulation feeding diversity across month, as well as to investigate the impacts of sex on intrapopulation feeding diversity. PS_i value closer to 1 indicates more generalist diet composition, and PS_i values closer to 0 indicate a specialist diet composition. Calculating PS_i for proportion diet data results in a metric of "biomass specialization" (Voelker et al. 2020) that can be used to describe whether predators individually specialize in biomass of specific prey items. This distinction is critical, as the biomass consumed does not equate to the number of individual prey items consumed. Given that scat samples were not collected from the same individual over time, due to the nature of wild scat collection, true individual diversity is not described. With these caveats, true individual biomass specialization cannot be defined, however, the PS_i metric can be used to compare

samples to each other across experimental groups, and can thus be used to describe intrapopulation diet diversity (Voelker et al. 2020).

Mathematically, PS_i calculations are bounded by a theoretical minimum and 1 (Voelker et al. 2020). For each population, the theoretical minimum is determined by one of two population specific values: the minimum prey density, or one over the sample size of the population (1/n). For the populations used within this study, the theoretical minimum was determined by sample size, as this value was consistently larger than the minimum prey density for all sample groups (Voelker et al. 2020). PS_i was calculated for each sample within sample groupings using the R package RInSp (Zaccarelli et al. 2013). Overall, average PS_i, as well as male average PS_i and female average PS_i was reported with 95% confidence intervals as determined by Monte Carlo bootstrapping simulations using the R package "resample". A logit transformation was then used on all individual PS_i values, since PS_i is bounded, which in turn shifts the variance distribution towards the mean and thus may obscure differences in PS_i (Bolnick et al. 2003, Voelker et al. 2020).

Correlations between prey species proportions and relative specialization

I calculated a correlation matrix using Spearman's correlation analysis of ranks to investigate the correlations between and 9 common prey species proportions (with overall season RRA >2%,) and PS_i values. Spearman's correlation was chosen as a Shapiro test showed that these data violate assumptions of normality. Positive correlations between prey species and PS_i show samples containing high proportions of that prey species correlate with samples containing higher PS_i values. Thus, a positive correlation indicates that a prey species is present in a more generalist diet. I then performed the same correlation analysis for male samples only, as well as

female samples only. I used the Benjamini-Hochberg procedure to adjust p-values and control false discovery rate for multiple comparisons testing (Benjamini and Hochberg 1995). Adjusted p-values were calculated in base R using the command “p.adjust”, where p values are ranked from small to large, then each p-value is multiplied by the total number of tests, and divided by its rank order. Significant adjusted p-values were set at $\alpha = 0.05$. Correlation analysis was run in R 4.2.0 using the ‘rstatix’ package (Kassambara 2021).

RESULTS

Sampling effort and analysis success

A total of 274 Steller sea lion scats were collected during December 2020-August 2021 from Tatoosh Island and Sea Lion Rock (Table 2). DNA metabarcoding was unsuccessful for 5 scats and 2 scats contained less than 20 prey reads, resulting in 267 scats used for relative read abundance (RRA) calculations. In addition, 11 scats lacked the presence of hard parts and were excluded from split sample frequency of occurrence analysis, resulting in 263 scats used in hard parts analysis (Table 2). 257 scats returned both hard parts and metabarcoding. By using custom designed Taqman assays, I was able to determine the sex of the scat depositor for 245 samples (89.4%) of all scat collected. Overall, 238 samples had successful sex determination as well as DNA metabarcoding.

Table 2. Number of scats collected from Steller sea lions for each season that were successfully used in DNA metabarcoding, sex determining qPCR and hard parts analysis.

		Scats collected	DNA metabarcoding	qPCR analysis	Hard parts recovery
Winter		**94**	**92**	**93**	**91**
	Tatoosh Island	94	92	93	91
Spring		**90**	**90**	**76**	**89**
	Tatoosh Island	60	60	50	59
	Sea Lion Rock	30	30	26	30
Summer		**90**	**85**	**76**	**83**
	Tatoosh Island	61	59	55	56
	Sea Lion Rock	29	26	21	27
Total		274	267	245	263

Steller sea lion diet: DNA metabarcoding and hard parts results

Analysis via DNA metabarcoding determined the relative read abundance (RRA, %) of 51 species-level prey item identifications within 22 family groups (Table 3). DNA metabarcoding was successful in identifying all sequences to a species level except for rockfishes (Sebastidae), where 81.25% of rockfish RRA was only recovered to the family level. Analysis of the same scats using hard parts analysis (split sample frequency of occurrence, SSFO) identified 39 prey groups, comprised of 19 species, 17 families, 2 class, and 1 order level identification (Table 4).

Most common prey species varied depending on method of prey identification, but both methods identified similar top prey families. The top prey families detected via metabarcoding analysis (>5% RRA) and averaged across all seasons were clupeids (Clupiedae) 41.6%, soles (Pleuronectidae) 12.2%, cods (Gadidae) 9.9%, rockfishes 8%, salmonids (Salmonidae) 6.7%, and dogfish (Squalidae) 5.5% (Table 3). The top prey families detected via hard parts analysis (>5% SSFO) were cods 29.3%, clupeids 22.9%, rockfishes 13%, dogfish 8.6%, salmon 6.5% and skates (Rajidae) 5% (Table 4).

Hard parts identification recovered 11 prey items within 8 prey families undetected by DNA metabarcoding, all under 1% of the SSFO. Specifically, East Pacific red octopus (*Octopus rubescens*) 0.9%, Pacific sand lance (*Ammodytes hexapterus or personatus*) 0.76% , opalecent squid (*Doryteuthis opalescens*) 0.6%, eelpouts (*Zoarcidae*) 0.5%, poachers (*Agnosis* spp.) 0.3%, three-spined stickleback (*Gasterosteus aculeatus*) 0.2%, plainfin midshipman (*Porichthys notatus*) 0.1%, unidentified mammals (class Mammalia) 0.1%, lefteye flounders (*Bothidae*) <0.1%, northern anchovy (*Engraulis mordax*) <0.1%, and shiner perch (*Cymatogaster aggregata*) <0.1%. DNA metabarcoding detected 39 species-level prey items not found in hard

parts analysis. Four of these species comprised over 1% of the overall diet as determined by RRA: big skate (*Raja binoculata*) 4.7%, Pacific cod (*Gadus macrocephalus*) 1.8%, arrowtooth flounder (*Atheresthes stomias*) 1.7%, dover sole (*Microstomus Pacificus*) 1.6%, sablefish (*Anoplopoma fimbria*) 1.4%. The families for all these species were also detected in hard parts recovery except for sablefish.

For some prey groups, the two methods detected different species. For example, cephalopods were detected in similar frequency across methods, hard parts contained 19 samples and DNA metabarcoding showed 18 samples with cephalopod DNA. However, DNA metabarcoding detected overall cephalopod RRA of 0.5%, completely comprised of giant Pacific octopus (*Enteroctopus dofleini*). Hard parts analysis detected 1.9% SSFO of cephalopods, comprised of unidentified cephalopods, opalecent squid (*Doryteuthis opalescens*), east Pacific red octopus (*Octopus rubescens*).

Species level identification of Sebastidae and Salmonidae specimen is not easily recovered via hard parts analysis, resulting in large differences between method recovery. Specifically, salmonids were detected in 63 (23.9%) samples via hard parts analysis and 128 (41.1%) samples via DNA metabarcoding analysis. Pacific salmon DNA and hard parts were both present in a total of 40 samples. DNA metabarcoding found 94 samples with >2 species of salmon present. Hard parts analysis showed one sample with >2 species of salmon. Five scats contained hard parts identified to salmon species. Of these samples, 3 were identified to coho salmon via hard parts and coho DNA comprised contained over 50% of these samples. Two samples containing Chinook salmon hard part remains, but contained no salmon DNA (Tables 3 and 4). Further, 27 samples contained steelhead (*Oncorhynchus mykiss*) DNA, but were completely absent of salmonid hard parts.

Table 3. Relative read abundance (%) of prey species recovered via DNA metabarcoding analysis in Steller sea lion scats in northwest Washington State. Prey items grouped by prey family and species.

Season	Overall	Winter	Spring	Summer
Sample size	267	92	90	85
Prey family Species				
Herrings, Shads, Sardines - Clupeidae	41.6	38.7	38.6	47.1
Pacific herring (*Clupea pallasii*)	24.8	12.8	37.0	25.4
American shad (*Alosa sapidissima*)	16.7	25.9	1.7	21.7
Pacific sardine (*Sardinops sagax*)	<0.1	<0.1	0.0	<0.1
Flatfishes - Pleuronectidae	12.2	5.7	30.8	1.5
Starry flounder (*Platichthys stellatus*)	6.2	1.1	18.2	0.0
English sole (*Parophrys vetulus*)	2.6	3.9	3.1	0.9
Arrowtooth flounder (*Atheresthes stomias*)	1.7	0.4	4.8	0.0
Dover sole (*Microstomus Pacificus*)	1.6	<0.1	4.5	0.3
Flathead sole (*Hippoglossoides elassodon*)	0.1	<0.1	0.0	0.3
Rex sole (*Glyptocephalus zachirus*)	<0.1	0.2	0.0	<0.1
Pacific halibut (*Hippoglossus stenolepis*)	<0.1	0.0	<0.1	0.0
Butter sole (*Isopsetta isolepis*)	<0.1	0.0	<0.1	0.0
Cods - Gadidae	9.9	11.7	8.3	9.7
Walleye pollock (*Gadus chalcogrammus*)	7.9	10.7	5.5	7.5
Pacific cod (*Gadus macrocephalus*)	1.8	0.9	2.4	2.2
Pacific tomcod (*Microgadus proximus*)	0.1	<0.1	0.4	0.0
Rockfishes - Sebastidae	8.0	5.5	3.5	14.5
Sebastes spp. (*Sebastes spp.*)	6.5	3.8	2.9	12.4
Widow rockfish (*Sebastes entomelas*)	0.8	1.7	0.0	0.6
Green striped rockfish (*Sebastes elongatus*)	0.2	0.0	0.0	0.7
Shortspine thornyhead (*Sebastolobus alascanus*)	0.2	<0.1	0.0	0.5
Canary rockfish (*Sebastes pinniger*)	0.1	<0.1	0.1	0.1
Yelloweye rockfish (*Sebastes ruberrimus*)	<0.1	0.0	0.0	0.2
Salmon - Salmonidae	6.7	7.8	4.2	7.8
Chinook salmon (*Oncorhynchus tshawytscha*)	2.3	3.6	0.5	2.7
Coho salmon (*Oncorhynchus kisutch*)	2.2	2.3	0.3	4.0
Pink salmon (*Oncorhynchus gorbuscha*)	1.4	1.0	2.9	0.3
Steelhead (*Oncorhynchus mykiss*)	0.7	1.0	0.3	0.8
Sockeye salmon (*Oncorhynchus nerka*)	<0.1	0.0	0.2	0.0

Chum salmon (*Oncorhynchus keta*)	<0.1	0.0	0.0	<0.1
Brown trout (*Salmo trutta*)	<0.1	<0.1	0.0	0.0
Skates - Rajidae	5.8	12.1	3.0	2.1
Big skate (*Raja binoculata*)	4.7	10.9	2.9	0.2
Sandpaper skate (*Bathyraja interrupta*)	0.6	0.6	0.0	1.1
Longnose Skate (*Raja rhina*)	0.5	0.6	0.1	0.9
Dogfish sharks - Squalidae				
Pacific spiny dogfish (*Squalus suckleyi*)	5.5	5.8	2.9	7.7
Greenlings - Hexagrammidae	3.3	2.9	1.0	5.8
Lingcod (*Ophiodon elongatus*)	2.9	2.7	0.9	4.9
Kelp greenling (*Hexagrammos decagrammus*)	0.4	0.2	0.1	0.9
Hakes - Merlucciidae				
Pacific hake (*Merluccius productus*)	1.7	2.5	2.8	0.0
Sablefishes - Anoplopomatidae				
Sablefish (*Anoplopoma fimbria*)	1.4	1.0	3.1	<0.1
Sculpins - Cottidae	1.2	1.7	0.7	1.1
Brown irish lord (*Hemilepidotus spinosus*)	0.8	1.1	0.2	1.1
Cabezon (*Scorpaenichthys marmoratus*)	0.2	0.4	0.2	<0.1
Pacific staghorn sculpin (*Leptocottus armatus*)	<0.1	0.2	<0.1	0.0
Sand flounders - Paralichthyidae				
Pacific sanddab (*Citharichthys xanthostigma*)	0.7	1.2	0.6	0.3
Ratfishes - Chimaeridae				
Spotted ratfish (*Hydrolagus colliei*)	0.7	0.7	0.0	1.3
Octopuses - Enteroctopodidae				
Giant Pacific octopus (*Enteroctopus dofleini*)	0.5	0.6	0.2	0.6
Smelts - Osmeridae	0.3	0.7	0.1	0.1
Eulachon (*Thaleichthys Pacificus*)	0.2	0.5	0.1	0.1
Surf smelt (*Hypomesus pretiosus*)	<0.1	0.1	<0.1	0.0
Whitebait smelt (*Allosmerus elongatus*)	<0.1	<0.1	<0.1	0.0
Ragfish - Icosteidae				
Ragfish (*Icosteus aenigmaticus*)	0.3	0.8	0.0	0.0
Surfperches - Embiotocidae				
Redtail surfperch (*Amphistichus rhodoterus*)	0.2	0.5	0.0	0.0
Tube snouts - Aulorhynchidae				
Tube snout (*Aulorhynchus flavidus*)	<0.1	0.0	0.0	0.2
Sandfishes - Trichodontidae				
Pacific sandfish (*Trichodon trichodon*)	<0.1	<0.1	0.0	<0.1
Snail-fishes - Liparidae				
Slipskin snailfish (*Liparis fucensis*)	<0.1	0.0	0.0	<0.1
Wrymouths - Cryptacanthodidae				
Giant wrymouth (*Cryptacanthodes giganteus*)	<0.1	<0.1	0.0	0.0
Northern lamprey - Petromyzontidae				
Pacific lamprey (*Entosphenus tridentatus*)	<0.1	<0.1	0.0	0.0

Table 4. Split sample frequency of occurrence (%) of prey species recovered via hard parts analysis in Steller sea lion scats in northwest Washington State. Prey items grouped by family and species or lowest taxonomic group. Prey taxa marked with "cf" indicate confidence with genus identification and strong likelihood of species identification.

Season	Overall	Winter	Spring	Summer
Sample Size	264	87	90	87
Prey Groups Lowest Taxonomic Classification				
Cods - Gadidae	**29.2**	**25.3**	**35.0**	**27.1**
Walleye pollock (*Gadus chalcogrammus*)	17.8	12.9	25.3	15.0
Walleye pollock (*Gadus chalcogrammus*) – cf	1.5	0.6	1.1	3.0
Walleye pollock (*Gadus chalcogrammus*) – juvenile	1.0	0.9	1.5	0.6
Gadid, unidentified	6.0	4.6	6.2	7.2
Gadid, unidentified – cf	1.7	3.2	0.4	1.4
Gadid, unidentified - juvenile	0.3	0.8	0.0	0.0
Gadid, unidentified – adult	0.2	0.3	0.2	0.0
Pacific tomcod (*Microgadus proximus*)	0.6	1.3	0.4	0.0
Pacific tomcod (*Microgadus proximus*) – cf	0.2	0.6	0.0	0.0
Herrings, Shads, Sardines - Clupeidae	**22.9**	**27.7**	**21.6**	**19.3**
Pacific herring (*Clupea pallasii*)	10.9	17.0	5.4	10.2
Pacific herring (*Clupea pallasii*) – cf	1.8	0.8	1.3	3.3
Pacific herring (*Clupea pallasii*) – juvenile	0.1	0.0	0.3	0.0
Pacific herring (*Clupea pallasii*) – juvenile, cf	0.2	0.0	0.0	0.6
Pacific herring (*Clupea pallasii*) – adult	0.2	0.6	0.0	0.0
Clupeids, unidentified	6.3	4.4	10.1	4.4
Clupeids, unidentified - cf	0.3	0.3	0.6	0.0
Clupeids, unidentified - juvenile	0.3	0.0	0.6	0.2
American shad (*Alosa sapidissima*)	2.0	3.3	2.2	0.6
American shad (*Alosa sapidissima*) – cf	0.4	0.0	1.3	0.0
American shad (*Alosa sapidissima*) – juvenile	0.4	1.3	0.0	0.0
Rockfishes - Sebastidae	**12.9**	**16.7**	**14.6**	**7.2**
Rockfishes, unidentified (*Sebastes* spp.)	9.5	13.6	9.2	5.5
Rockfishes, unidentified (*Sebastes* spp.) – cf	1.7	1.8	1.7	1.7
Rockfishes, unidentified (*Sebastes* spp.)– adult	1.0	0.6	2.4	0.0
Rockfishes, unidentified (*Sebastes* spp.) – adult, cf	0.1	0.4	0.0	0.0
Rockfishes, unidentified (*Sebastes* spp.) – juvenile	0.4	0.3	0.9	0.0
Rockfishes, unidentified (*Sebastes* spp.) – juvenile, cf	0.1	0.0	0.4	0.0
Spiny dogfish (Squalus acanthias)	**7.8**	**7.4**	**5.0**	**11.1**
Spiny dogfish (*Squalus acanthias*)	7.8	7.4	5.0	11.1
Salmon - Salmonidae	**6.3**	**8.1**	**4.6**	**6.2**

Salmon, unidentified (*Oncorhynchus* spp.)	1.0	1.0	0.7	1.4
Salmon, unidentified (*Oncorhynchus* spp.) – cf	0.2	0.7	0.0	0.0
Salmon, unidentified (*Oncorhynchus* spp.) – juvenile	1.8	2.6	1.0	1.6
Salmon, unidentified (*Oncorhynchus* spp.) – adult	0.2	0.2	0.3	0.0
Salmon, unidentified (*Oncorhynchus* spp.) – adult, cf	0.1	0.0	0.4	0.0
Salmon, unidentified (excluding Chinook)	1.2	0.4	0.9	2.5
Salmon, unidentified (excluding Chinook) – juvenile	1.3	2.3	1.0	0.5
Coho salmon (*Oncorhynchus kisutch*)	0.1	0.3	0.0	0.0
Coho salmon (*Oncorhynchus kisutch*) – juvenile	0.1	0.0	0.4	0.0
Chinook salmon (*Oncorhynchus tshawytscha*)	0.1	0.0	0.0	0.2
Chinook salmon (*Oncorhynchus tshawytscha*) – juvenile	0.1	0.4	0.0	0.0
Salmon or trout, unidentified – adult	0.1	0.3	0.0	0.0
Skates - Rajidae	**5.1**	**5.5**	**7.2**	**2.6**
Skates, unidentified	5.1	5.5	7.2	2.6
Flatfishes - Pleuronectidae	**2.6**	**1.4**	**2.1**	**4.4**
Starry flounder (*Platichthys stellatus*)	1.1	0.0	1.5	1.8
Starry flounder (*Platichthys stellatus*) – cf	0.3	1.0	0.0	0.0
Flatfishes, unidentified	0.6	0.4	0.6	0.9
Flatfishes, unidentified – cf	0.5	0.0	0.0	1.5
English sole (*Parophrys vetulus*)	0.1	0.0	0.0	0.2
Greenlings – Hexagrammidae	**2.2**	**0.3**	**0.0**	**6.5**
Lingcod (*Ophiodon elongatus*)	0.6	0.3	0.0	1.4
Lingcod (*Ophiodon elongatus*) – cf	1.7	0.0	0.0	5.1
Order Cephalopoda	**1.9**	**1.5**	**2.8**	**1.4**
East Pacific red Octopus (*Octopus rubescens*)	0.8	0.5	1.1	0.7
East Pacific red Octopus (*Octopus rubescens*) – juvenile	0.1	0.3	0.0	0.0
Opalecent squid (*Doryteuthis opalescens*)	0.5	0.4	0.9	0.2
Opalecent squid (*Doryteuthis opalescens*) – juvenile	0.1	0.0	0.0	0.2
Cephalopod spp.	0.4	0.3	0.8	0.3
Class Elasmobranchii - Cartilaginous fishes	**1.8**	**0.0**	**1.5**	**4.0**
Elasmobranch, unidentified	1.3	0.0	1.0	2.9
Elasmobranch, unidentified – cf	0.4	0.0	0.0	1.1
Elasmobranch, unidentified – adult	0.2	0.0	0.6	0.0
Smelts - Osmeridae	**1.6**	**0.3**	**3.0**	**1.4**
Osmeridae spp.	1.1	0.3	2.6	0.2
Osmeridae spp. – cf	0.5	0.0	0.4	1.2
Hakes – Merlucciidae	**0.84**	**0.50**	**0.14**	**1.92**
Pacific Hake (*Merluccius productus*)	0.20	0.28	0.14	0.19
Pacific Hake (*Merluccius productus*) – cf	0.64	0.22	0.00	1.72
	0.76	**0.00**	**0.50**	**1.82**

Dogfish sharks - Squalidae				
Spiny dogfish (*Squalus acanthias*) – juvenile	0.64	0.00	0.50	1.44
Spiny dogfish (*Squalus acanthias*)	0.12	0.00	0.00	0.38
Sand lances - Ammodytidae	**0.76**	**0.56**	**0.36**	**1.38**
Pacific sand lance (*Ammodytes hexapterus*)	0.63	0.56	0.36	1.00
Pacific sand lance (*Ammodytes hexapterus*) – juvenile	0.12	0.00	0.00	0.38
Sculpins - Cottidae	**0.61**	**0.78**	**0.00**	**1.05**
Irish lord, unidenfied (*Hemilepidotus* spp.)	0.31	0.56	0.00	0.38
Pacific Staghorn Sculpin (*Leptocottus armatus*)	0.22	0.00	0.00	0.67
Sculpins, unidentified – juvenile	0.07	0.22	0.00	0.00
Eelpouts - Zoarcidae	0.52	0.22	0.00	1.36
Eelpouts, unidenfied	0.34	0.22	0.00	0.80
Eelpouts, unidenfied – cf	0.11	0.00	0.00	0.33
Eelpouts, unidenfied – juvenile cf	0.07	0.00	0.00	0.23
Lampreys - Petromyzontidae	**0.51**	**0.83**	**0.14**	**0.57**
Lamprey, unidentified	0.51	0.83	0.14	0.57
Snail fishes - Liparidae	**0.37**	**0.93**	**0.16**	**0.00**
Snail fishes, unidentified	0.05	0.00	0.16	0.00
Snail fishes, unidentified – cf	0.19	0.56	0.00	0.00
Snail fishes, unidentified – juvenile	0.12	0.37	0.00	0.00
Poachers - Agonidae	**0.32**	**0.67**	**0.28**	**0.00**
Poacher, unidentified (*Agonopsis* spp.)	0.32	0.67	0.28	0.00
Gunnels - Pholidae	**0.22**	**0.67**	**0.00**	**0.00**
Gunnel, unidentified	0.22	0.67	0.00	0.00
Sticklebacks - Gasterosteidae	**0.19**	**0.00**	**0.56**	**0.00**
Three spined stickleback (*Gasterosteus aculeatus*)	0.19	0.00	0.56	0.00
Batrachoididae	**0.13**	**0.22**	**0.16**	**0.00**
Plainfin midshipman (*Porichthys notatus*)	0.05	0.00	0.16	0.00
Plainfin midshipman (*Porichthys notatus*) – cf	0.07	0.22	0.00	0.00
Class Mammalia	**0.12**	**0.37**	**0.00**	**0.00**
Mammal, unidentified	0.12	0.37	0.00	0.00
Paralichthyidae	**0.12**	**0.00**	**0.00**	**0.38**
Sanddab spp.	0.12	0.00	0.00	0.38
Anchovies - Engraulidae	**0.07**	**0.00**	**0.22**	**0.00**
Northern anchovy (*Engraulis mordax*) – cf	0.07	0.00	0.22	0.00
Surfperches - Embiotocidae	**0.07**	**0.22**	**0.00**	**0.00**
Shiner perch (*Cymatogaster aggregata*)	0.07	0.22	0.00	0.00
Lefteye Flounders - Bothidae	**0.05**	**0.00**	**0.00**	**0.16**
Flounder, unidentified – cf	0.05	0.00	0.00	0.16

Comparing observed sex demographics with qPCR analysis

Observed sex counts were collected for six of the seven months where Tatoosh Island was sampled: December, January, February, March, June and July (Figure 2). Juvenile Steller sea lions comprised most of the population demographic, accounting for at least 40% of all monthly counts (Figure 2). Adult female Steller sea lions were higher in abundance than adult males during the winter and spring months and adult males were higher in the summer (Figure 2). Using the expected ratio of male to female juveniles, the overall observed ratio showed similar trends, with higher females observed December through March, and lower females observed June and July (Figure 3).

A total of 238 scats had successful DNA metabarcoding and qPCR sex determination, and were used in subsequent analysis (Table 5). A higher proportion of females as detected by qPCR analysis occurred in winter and spring, followed by increased males detected in the summer (Table 4). A Pearson's correlation analysis showed a significant positive correlation (Figure 5, $t_4=4.26$, $p = 0.013$, $R=0.91$) between the proportion of male Steller sea lions determined via observed counts (Figure 3) and the proportion of male Steller sea lions determined via qPCR analysis of scat (Figure 4).

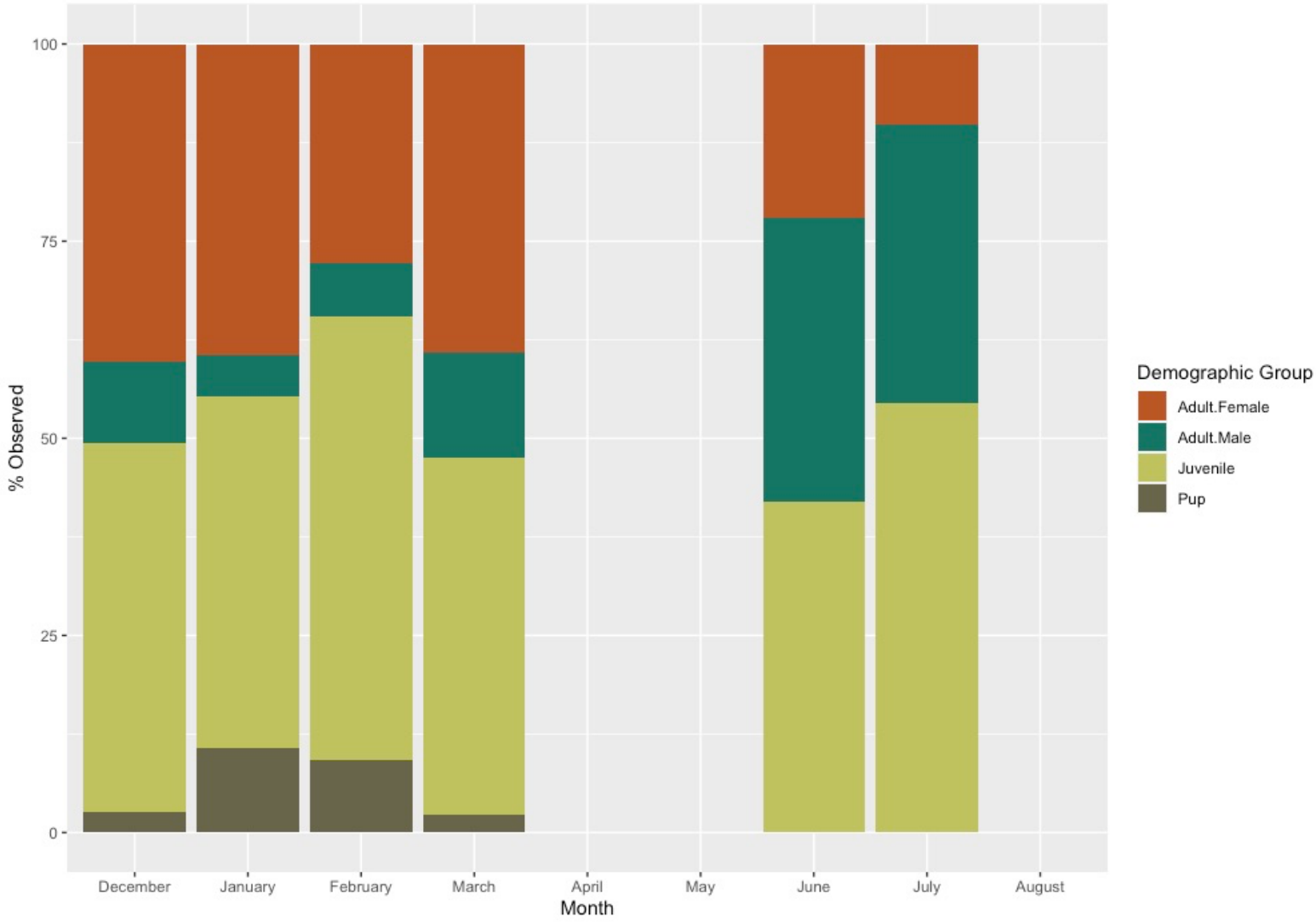

Figure 2. Observed percentage of counts of Steller sea lion demographic groups based on sex and age, based on observational counts of adult males, adult females, juveniles and pups at Tatoosh Island.

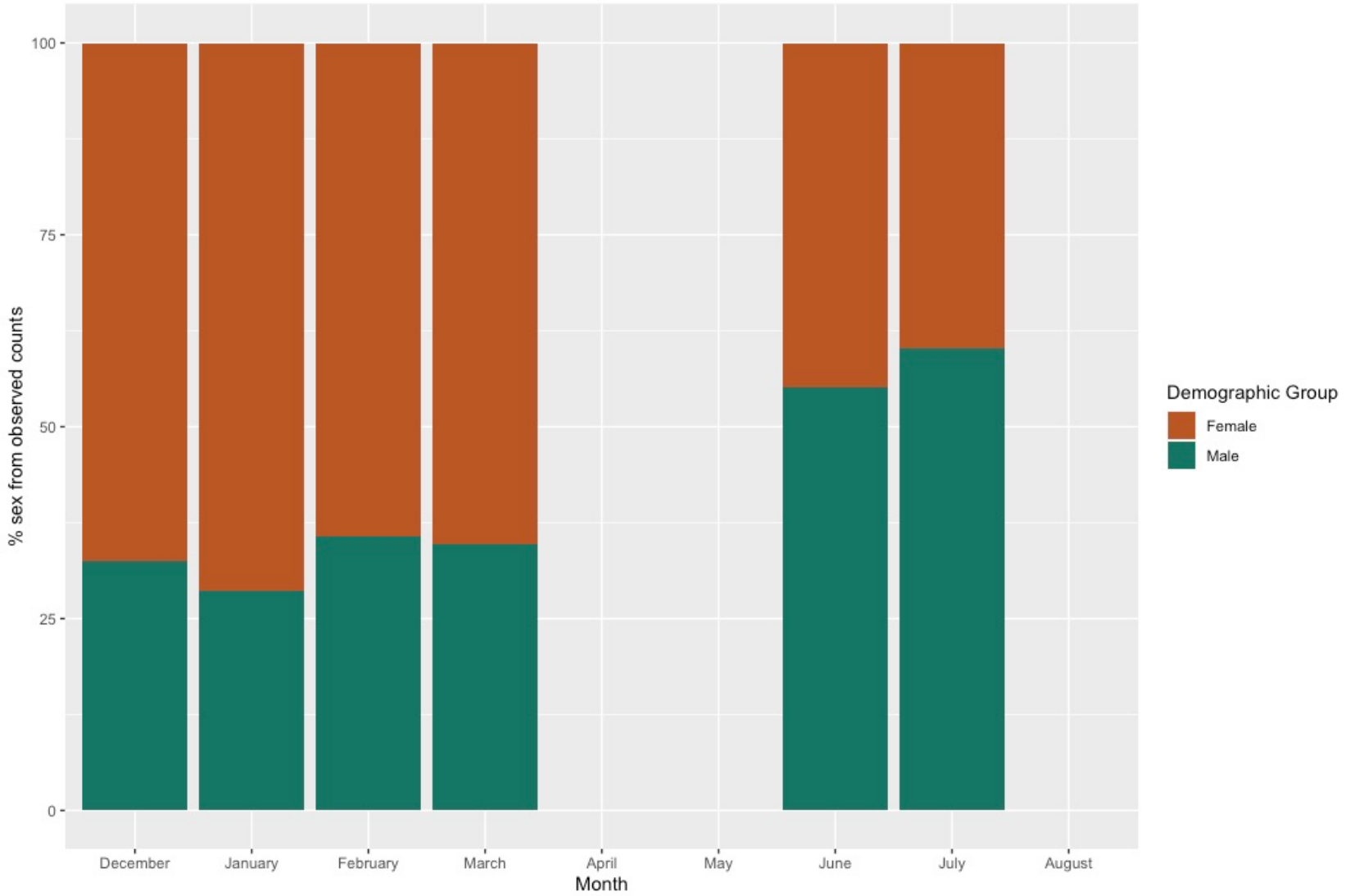

Figure 3. Observed percentage of Steller sea lion sex regardless of age, with juvenile sex estimated by expected sex-proportion of juvenile Steller sea lions as 54.4% female and 45.6% male. Pup counts were excluded from estimates.

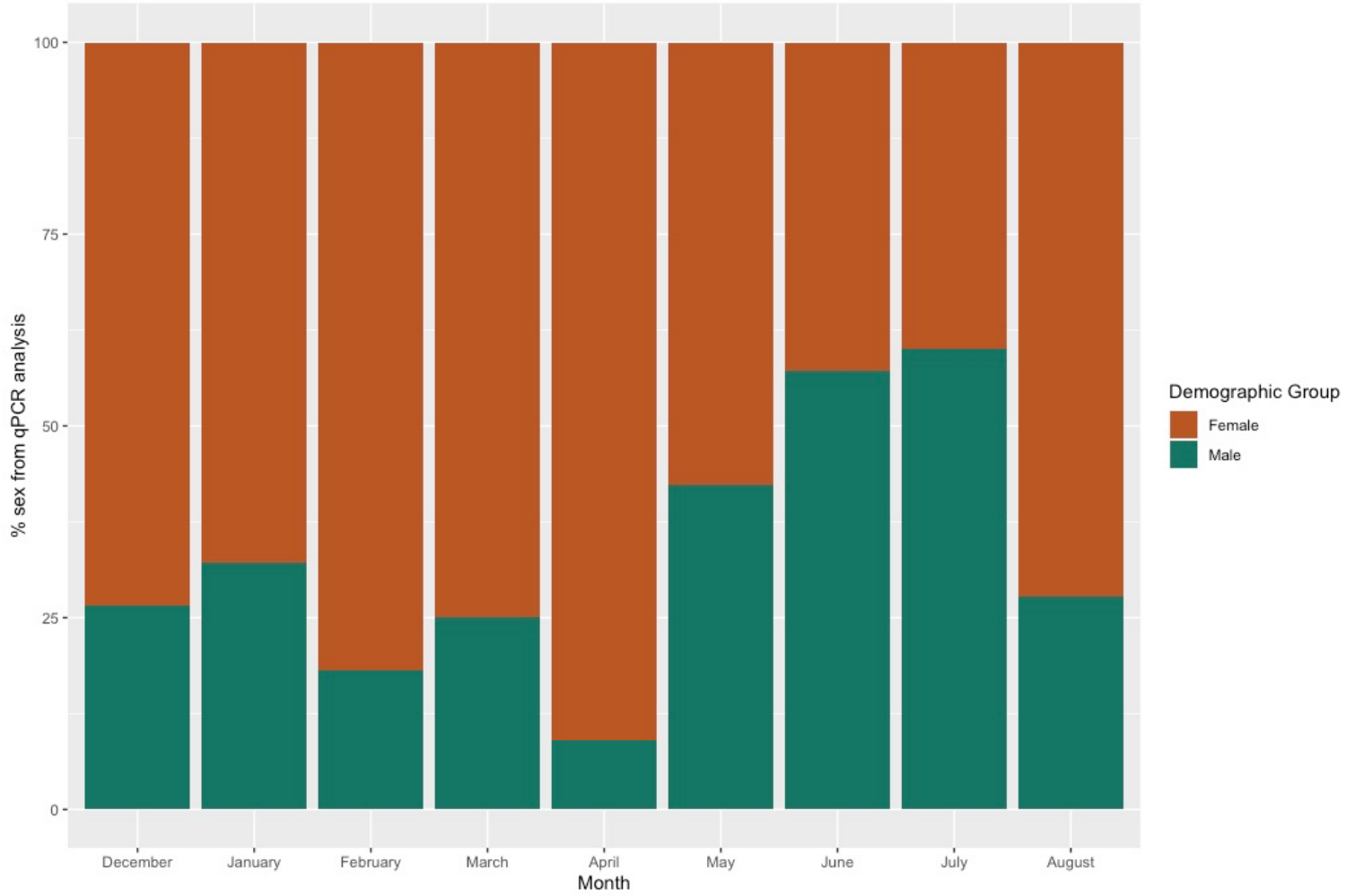

Figure 4. Monthly percentage of scat samples that were from male and female Steller sea lions in northwest Washington during 2020-2021 as determined by qPCR analysis.

Table 5. Counts of sexed Steller sea lion scat from each season that had successful DNA metabarcoding analysis (n=238) and proportion of male scats recovered per sample group as determined by qPCR analysis.

Season	Site	Total sexed scats with DNA metabarcoding	Female	Male
Winter		**91**	**68**	**23**
	Tatoosh Island	91	68	23
Spring		**76**	**56**	**20**
	Tatoosh Island	50	41	9
	Sea Lion Rock	26	15	11
Summer		**71**	**35**	**36**
	Tatoosh Island	53	22	31
	Sea Lion Rock	18	13	5
Total		238	159	79

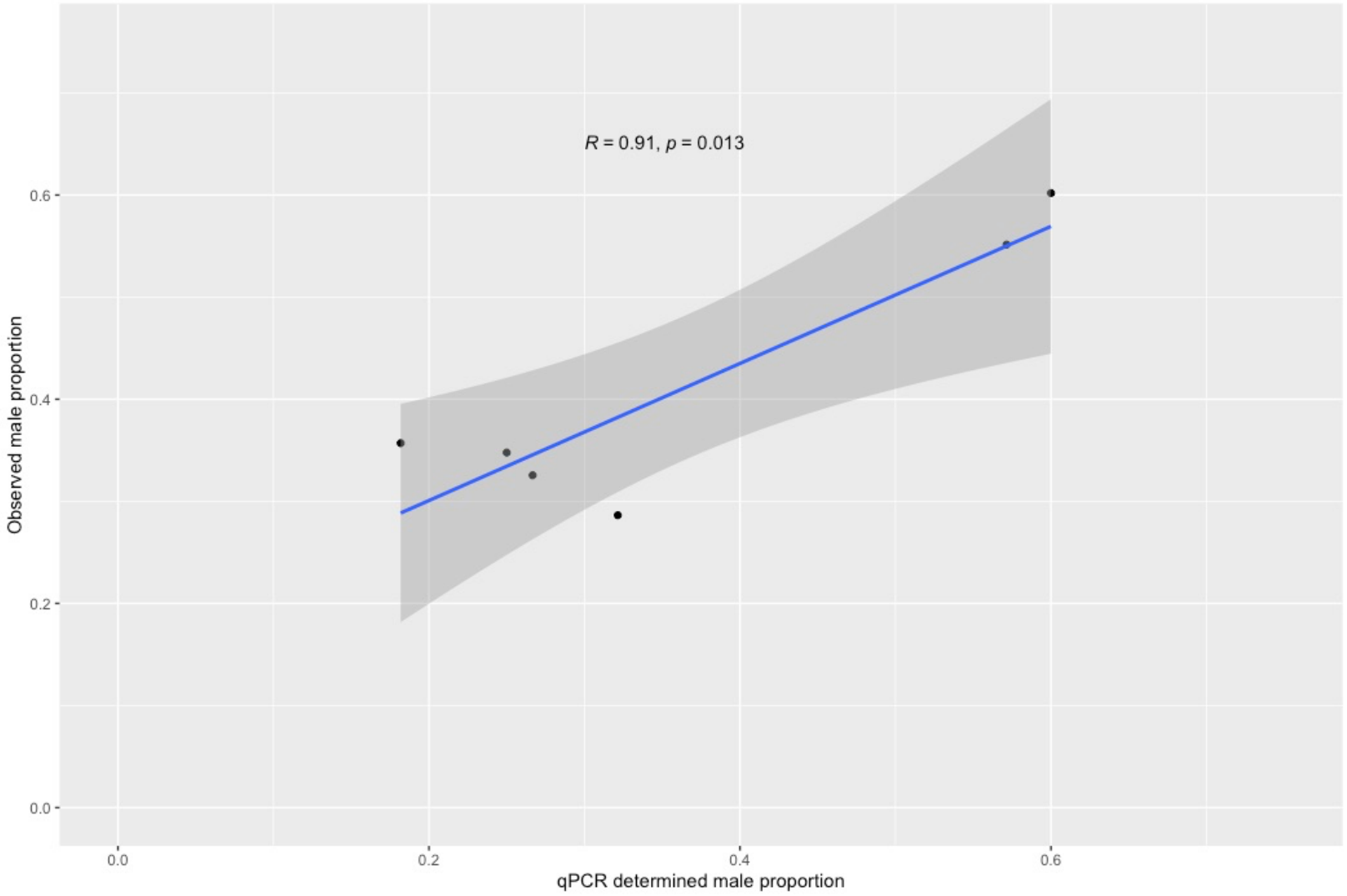

Figure 5: Correlation plot comparing male Steller sea lions as determined by observed haulout counts against the male proportion of Steller sea lion scats determined via qPCR analysis. Shaded region represents 95% confidence intervals.

DNA metabarcoding diet composition across seasons and sex

Steller sea lion diet, as determined via relative read abundance (RRA), showed significant variation in diet composition across seasons (Table 3). Individual scat samples showed homogenous dispersion within seasonal groups, indicating that within season variation in diet was similar (betadisper, permutation test; $p = 0.946$). A subsequent PERMANOVA of all species RRA showed that season was significant, yet only explains 8.89% of the variation in diet composition (Figure 6, PERMANOVA: $r2 = 8.89\%$, $p < 0.00001$). Individual scat samples showed heterogenous dispersion within monthly groups, indicating that within month variation in diet was not similar (betadisper, permutation test; $p = 0.001$). Given that sample size was not equal across all months (ranging from sample sizes of 29-34 scats per sampling unit) and that dispersion was unequal across months, monthly changes in individual diet composition could not be compared using a PERMANOVA (Anderson and Walsh 2013). However, visual representation of diet composition shows monthly variation (Figure 7).

I used additional dispersion tests and PERMANOVAs to investigate the impacts of sex on diet composition. I found that dispersion was not homogenous within samples grouped by season and sex (betadisper, permutation test; $p = 0.01$). Further, I found that dispersion was not homogenous within samples grouped by month and sex (betadisper, permutation test; $p = 0.001$). Therefore, I investigated the effect of sex separately for each season (Figure 6). Analysis of dispersion for winter and spring showed that males and females were equally dispersed (betadisper, permutation tests; $p=0.4407$ and $p = 0.051$, respectively), and thus diet variation was equal across both sexes within these seasons. During summer, male diet was slightly more dispersed relative to female diet (Tukeys HSD: Male-Female difference = 0.124, lower 95% CI =

0.019, upper 95% = CI 0.228, p = 0.02), indicating a higher variation in diet composition among males in the summer. PERMANOVAs of winter and spring diet data showed no significant impact of sex on diet composition (r2= 1.7%, p = 0.153 and r2 = 1.8%, p = 0.519, respectively). Despite overdispersion, the equal balance of male (n= 36) and female scats collected (n=35) in the summer make PERMANOVA an acceptable choice for analysis (Anderson and Walsh 2013). PERMANOVA of summer diet data further showed sex had no significant impact of diet composition (r2= 2.3%, p = 0.149).

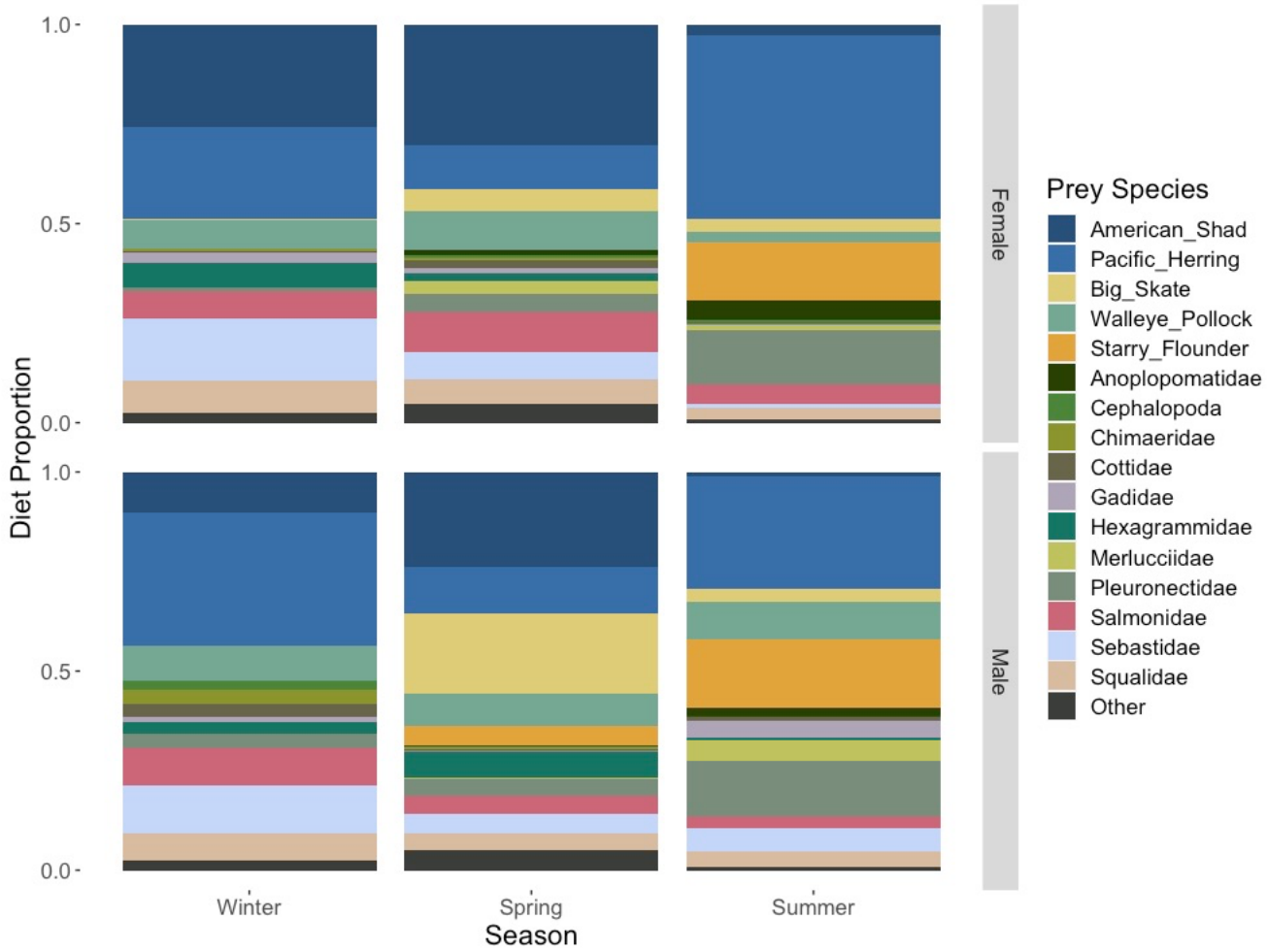

Figure 6. Average relative read abundance (RRA) of prey families and top five species (>5% RRA in at least one season) recovered via DNA metabarcoding from Steller sea lion scats relative to sea lion sex during winter 2020, spring 2021 and summer 2021.

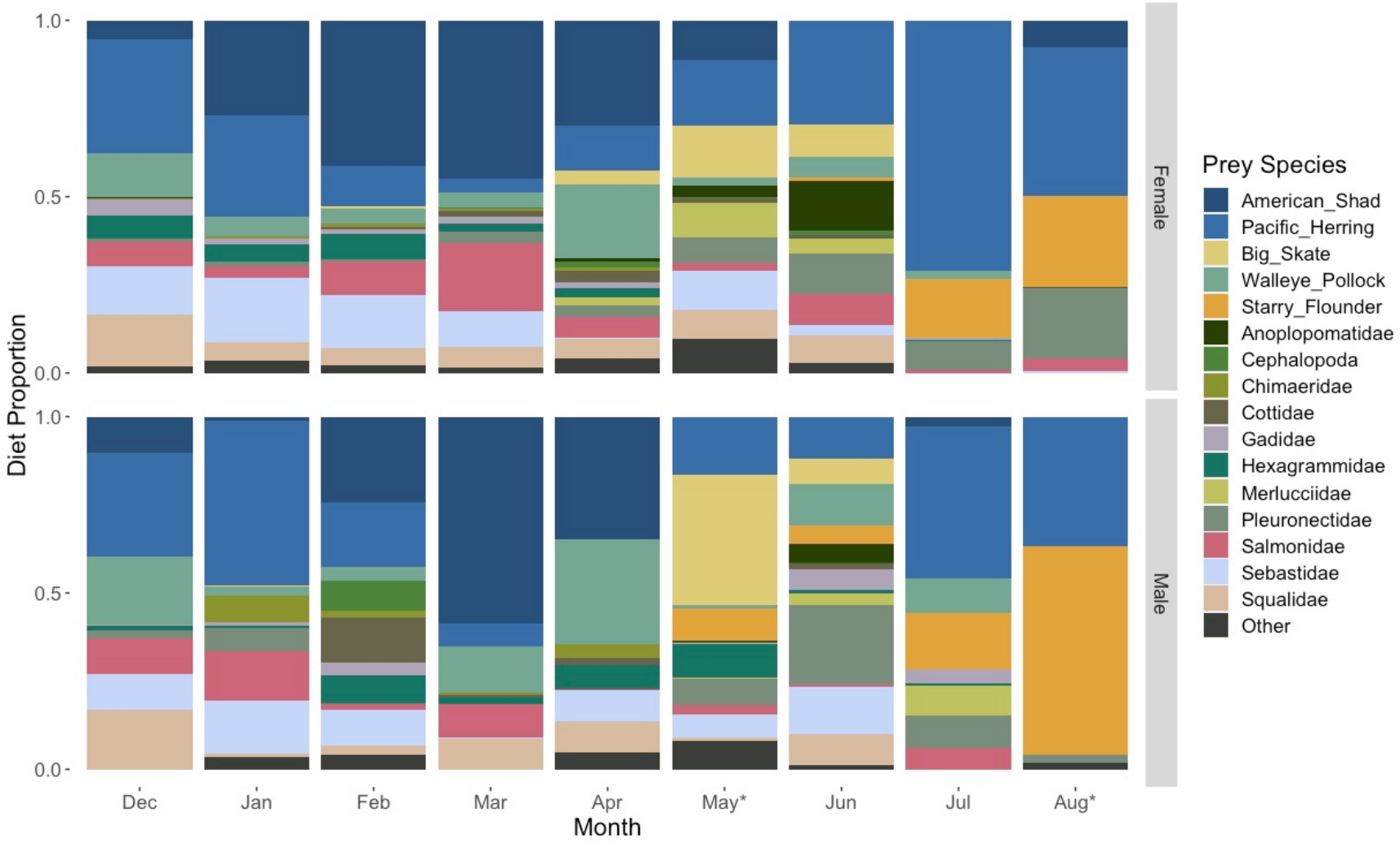

Figure 7. Average relative read abundance (RRA) of prey families and top five species (>5% RRA in at least one season) recovered via DNA metabarcoding from Steller sea lion scats relative to sea lion sex during December 2020- August 2021. Months marked with asterisk indicate months where diet data was collected from Sea Lion Rock haulout. All other months were collected from the Tatoosh Island haulout complex.

Steller sea lion population diet diversity and relative individual diet specialization

Shannon diet diversity indices (H) ranged from 2.03 to 2.59 and showed visual trends in diet diversity of Steller sea lions depending on season and sex (Figure 8). In the winter, males had a higher Shannon diet diversity indices than females (winter, males H=2.41, females H=2.24) Higher male diet diversity is likely due to Differences due to season in the winter were due to the presence of cephalopods, cottids and gadids present in male winter diets but absent in female winter diets (Figures 6 and 7). During the spring, males consistently showed lower diet diversity than females (spring, males H= 2.43, females H=2.59). In the summer, overall calculations of diet diversity show that the male Steller sea lion population displayed broader diet diversity than female (summer, males H= 2.42, females H=2.03). Across all seasons, male Shannon diet diversity indices remained consistent, from 2.41-2.43, whereas female Shannon diet diversity indices ranged from 2.03-2.59.

PSi values were calculated by Steller sea lion population groups defined by month (Table 6). PERMANOVA showed that diet composition significantly varied by season, but did not significantly vary based on sex. Visual differences in RRA (Figure 7) suggest that Steller sea lion diet varied monthly, and likely due to different prey availability. The average PSi of Steller sea lion samples was 0.397 (95% CI = 0.023, R = 10,000). The average PSi of only male Steller sea lion samples was 0.302 (95% CI = 0.041). The average PSi of only female Steller sea lion samples was 0.347 (95% CI = 0.028). Logit-transformed PSi values compared across month and sex showed little variation in sex (Figure 9), as the 95% confidence intervals of average logit PSi values overlapped between all sites and season.

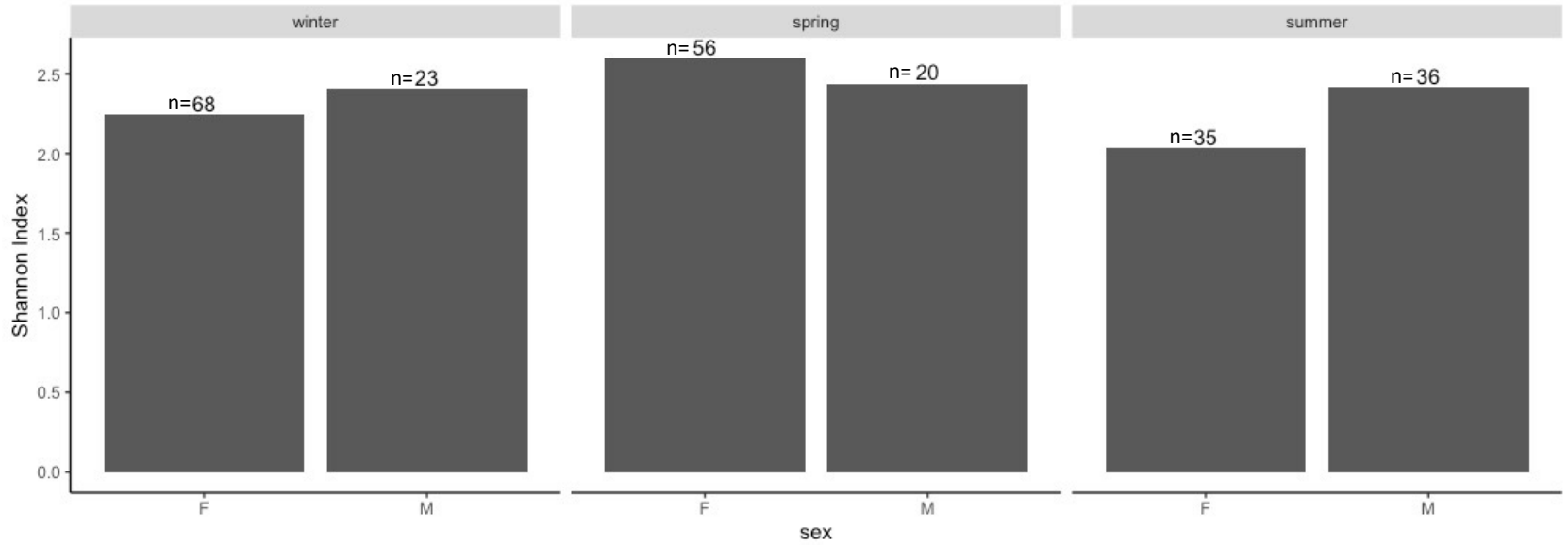

Figure 8. Shannon diet diversity indices (H) calculated from average species diet proportions (RRA) of Steller sea lions grouped by season and sex. Numbers above bar chart represent sample size of analysis pool.

Table 6. Sample population groupings used to calculate Proportional Similarity Indices (PS_i) and their average PS_i values for individual scats collected from Steller sea lions. Theoretical minimum for PS_i calculations are reported and determined by sample size (1/n).

Month	n	Theoretical min (1/n)	avg PS_i
December	30	0.0333	0.253
January	28	0.0357	0.285
February	33	0.0303	0.273
March	28	0.0357	0.401
April	22	0.0455	0.244
May	26	0.0385	0.207
June	28	0.0357	0.158
July	25	0.04	0.407
August	18	0.0555	0.370

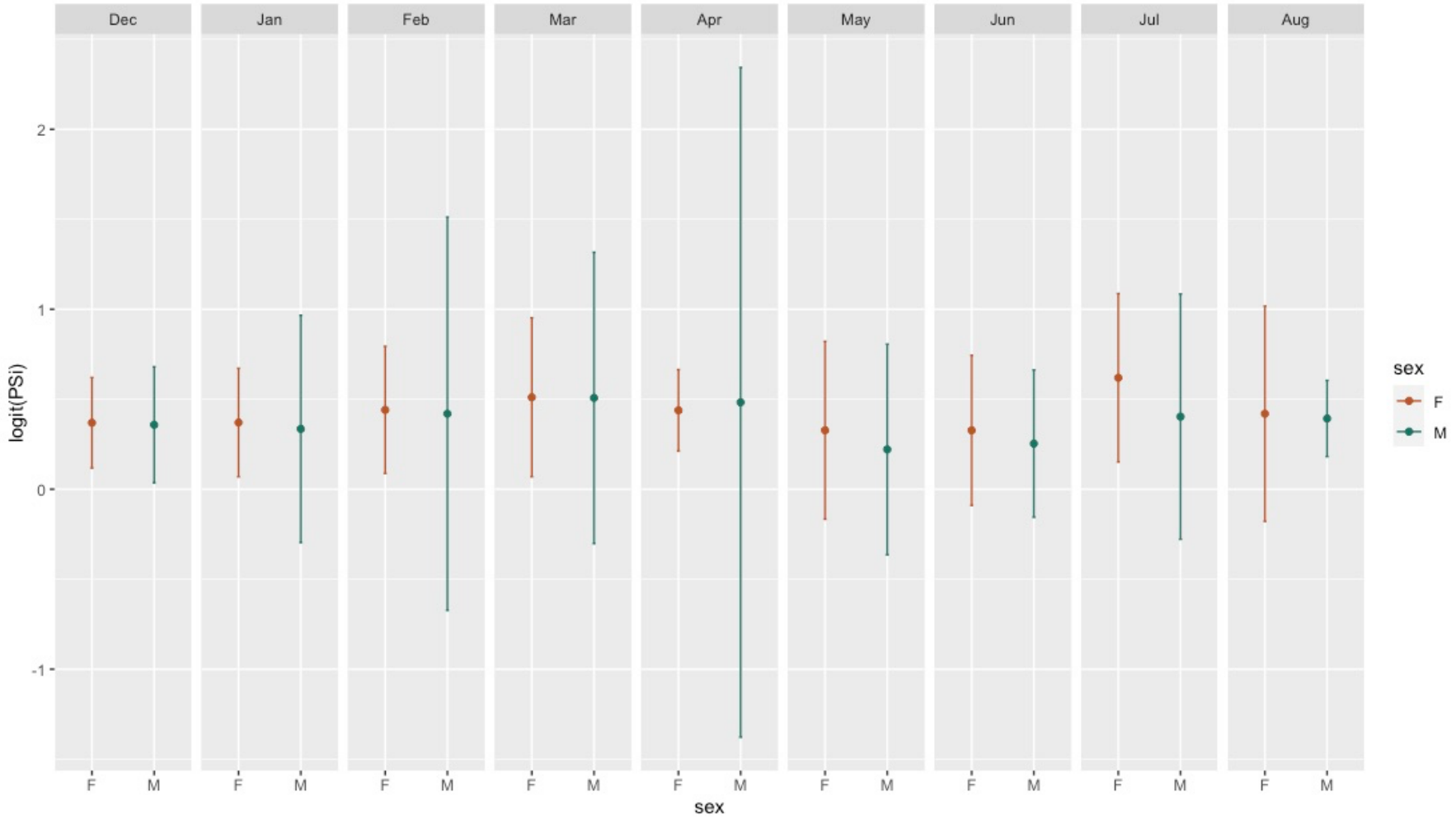

Figure 9. Logit-transformed average PS_i values with 95% confidence intervals of Steller sea lion scats (n=238 scats with sex determination and DNA metabarcoding). Individual PS_i values were then grouped by sex and month. Sample sizes and population groupings are described in Table 7.

Correlations between species and relative individual specialization

A Spearman's rank correlation analysis matrix between 9 common prey species (>2% RRA within a season, Table 7) and PS_i showed significant positive correlations (following Benjimani-Hochberg p value adjustment) between 5 top prey species, Pacific herring (rho = 0.31), American shad (rho =0.38), walleye pollock (rho = 0.25), Chinook salmon (rho = 0.26), Coho salmon (rho =0.33), starry flounder (rho = 0.13) and generalist diets (Figure 9). All species are considered semi-pelagic, and 3 of the five species are anadromous (Table 7). Big skate was the only species that correlated with specialist PS_i values (rho = -0.15). The correlation analysis also found 12 significant positive correlations between species, indicating high proportions of species occurring with each other (Figure 10, represented in blue). This analysis also found 20 significant negative correlations between species (Figure 10, represented in red). The strongest significant negative correlation is between two species within the clupeid family: American shad and Pacific herring (rho = -0.36).

I grouped samples by sex to further explore correlations between species and relative individual specialization. Correlation analysis performed with only female scat samples was similar to the correlations with both sexes, but showed a few differences (Figure 11). Pacific herring and spiny dogfish showed significant negative correlations in only female scats, however the trend was weak (-0.16). Many correlations found in the overall population were no longer significant in the female only dataset, although all absent trends showed weak correlations between rho -0.2 and 0.2. Correlation analysis performed with only male scat samples showed absence of significant correlations between generalist diets and Chinook salmon and coho salmon (Figure 12). Between species analysis of only male scats revealed positive correlation

between spiny dogfish and two species: lingcod (rho = 0.28) and Chinook salmon (rho =0.22) (Figure 12).

Table 7. Top prey species, as defined as >2% of relative read abundance, and habitat characteristics (Allen and Smith 1988), as well as highest seasonal abundance within Steller sea lion diet.

Species	Habitat	Overall RRA	Highest seasonal RRA
Pacific herring (*Clupea pallasii*)	Pelagic-neritic	24.80%	Spring, 37%
American shad (*Alosa sapidissima*)	Pelagic-neritic, anadromous	16.70%	Winter, 25.9%
Walleye pollock (*Gadus chalcogrammus*)	Benthopelagic	7.90%	Winter, 10.7%
Starry flounder (*Platichthys stellatus*)	Pelagic-neritic	6.20%	Spring, 18.2%
Spiny dogfish (*Squalus suckleyi*)	Benthopelagic	5.50%	Summer, 7.7%
Big skate (*Raja binoculata*)	Demersal	4.70%	Winter, 10.9%
Lingcod (*Ophiodon elongatus*)	Demersal	2.90%	Summer, 4.9%
English sole (*Parophrys vetulus*)	Demersal	2.60%	Winter, 3.9%
Chinook salmon (*Oncorhynchus tshawytscha*)	Benthopelagic, anadromous	2.30%	Winter, 3.6%
Coho salmon (*Oncorhynchus kisutch*)	Pelagic, anadromous	2.20%	Summer, 4.0%

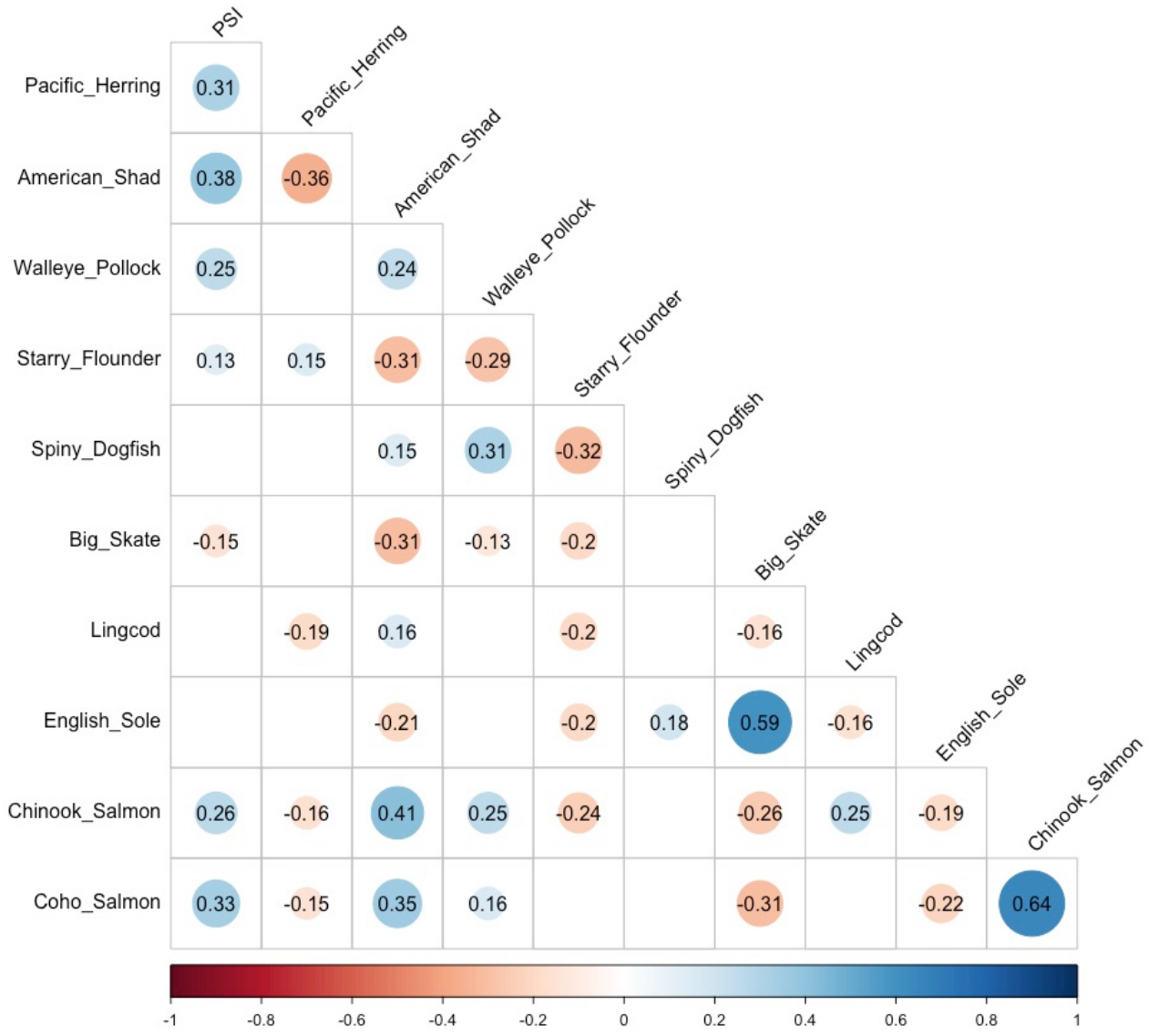

Figure 10. Correlation matrix of all Steller sea lion scat samples with DNA metabarcoding and sex determination (n=238) showing all significant correlations (α=0.05, Benjimani Hochberg p-adjustment) of 9 species that comprise >2% RRA of the Steller sea lion diet. Positive correlations are represented in blue and negative correlations in red, and rho values are presented within the matrix. Positive relationships with individual PS_i represent species correlating with more generalist diets and negative with more specialist diets. Positive relationships between species indicate co-occurrence of species within diets. Strength of correlation is indicated by rho values and size of matrix circles.

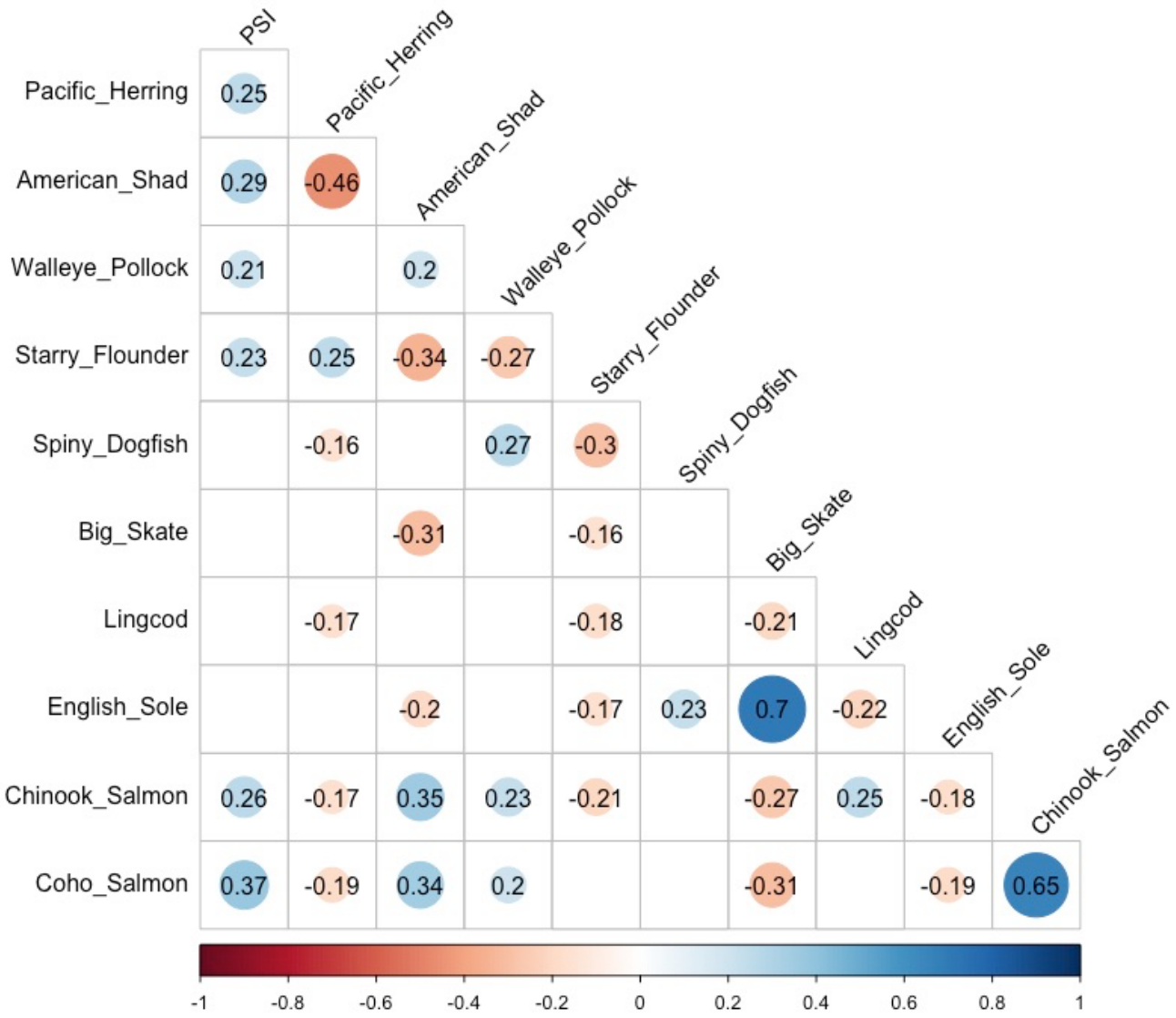

Figure 11. Correlation matrix of female Steller sea lion scat samples with DNA metabarcoding and sex determination (n=159) showing all significant correlations (α=0.05, Benjimani Hochberg p-adjustment) of 9 species that comprise >2% RRA of the Steller sea lion diet.. Positive correlations are represented in blue and negative correlations in red, and rho values are presented within the matrix. Positive relationships with individual PS_i represent species correlating with more generalist diets and negative with more specialist diets. Positive relationships between species indicate co-occurrence of species within diets. Strength of correlation is indicated by rho values and size of matrix circles.

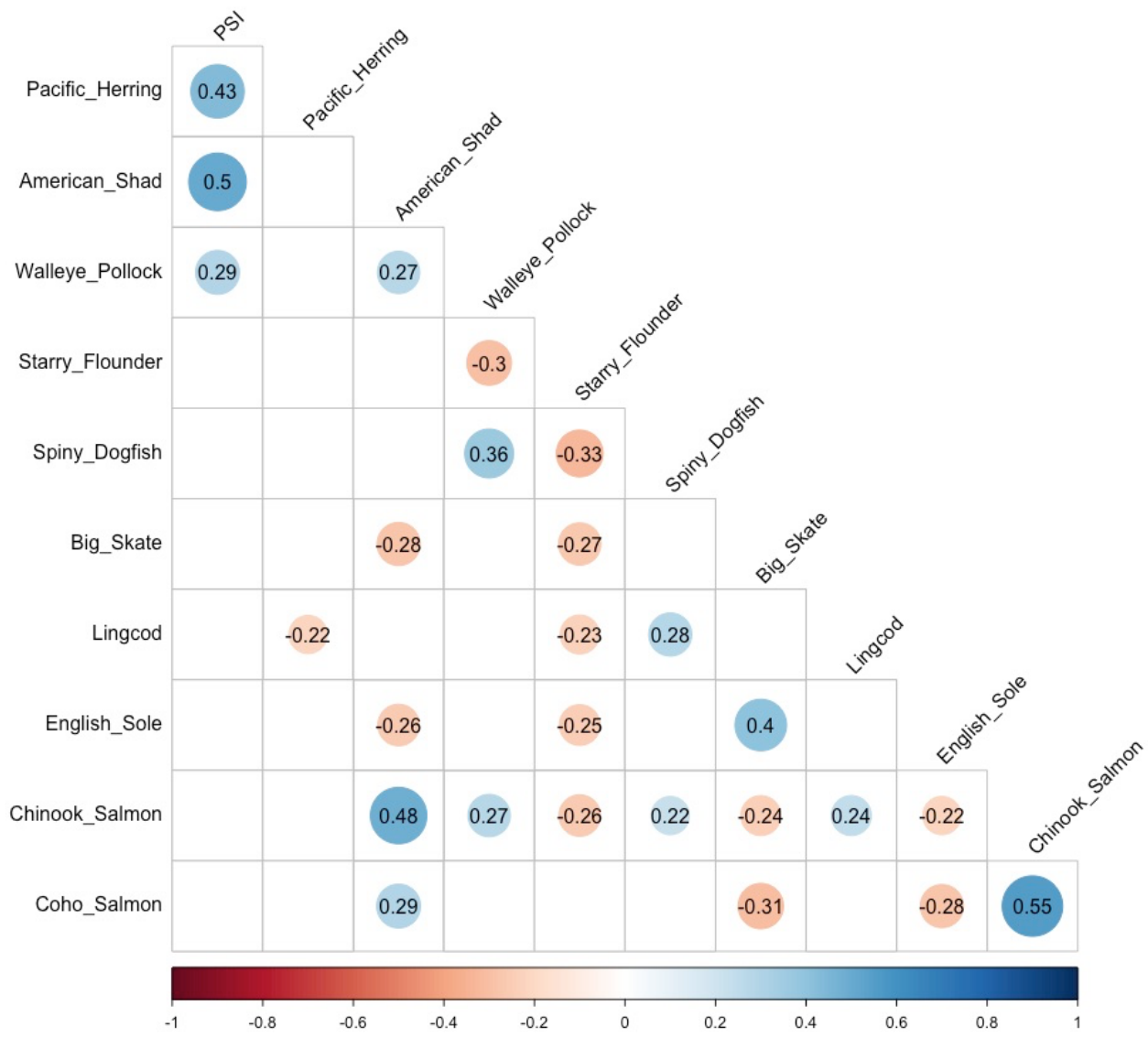

Figure 12. Correlation matrix of male Steller sea lion scat samples with DNA metabarcoding and sex determination (n=79) showing all significant correlations (α=0.05, Benjimani Hochberg p-adjustment) of 9 species that comprise >2% RRA of the Steller sea lion diet. Positive correlations are represented in blue and negative correlations in red, and rho values are presented within the matrix. Positive relationships with individual PS_i represent species correlating with more generalist diets and negative with more specialist diets. Positive relationships between species indicate co-occurrence of species within diets. Strength of correlation is indicated by rho values and size of matrix circles.

DISCUSSION

Steller sea lion diet along coastal Washington

I studied the diet of Steller sea lions (*Eumetopias jubatus*) on the northwest Washington coast between December 2020 through August 2021 and found that their diet, as determined by DNA metabarcoding, was dominated by Pacific herring (*Clupea pallasii*), American shad (*Alosa sapidissima*), walleye pollock (*Gadus chalcogrammus*), spiny dogfish (*Squalus suckleyi*), rockfishes (*Sebastes* spp.), and starry flounder (*Platichthys stellatus*) (RRA >5%, Figure 6). Together, these prey groups comprise over 50% of Steller sea lion diet in all seasons (Table 3, Figures 6 and 7). Clupeidae, containing both Pacific herring (hereafter referred to as herring) and American shad (hereafter referred to as shad), were the dominant prey family in Steller sea lion diet year-round (winter 38.7%, spring, 38.6%, and summer 47.1%).

The top prey items found in the diet of Steller sea lions along the coast of Washington are similar to those in other regions of the eastern North Pacific. Most frequent prey items of Steller sea lion diet in northern California and Oregon from 1986-2007 were Pacific hake, Pacific salmon, skate, Pacific lamprey, rockfish and clupeids (Riemer et al. 2011). Steller sea lion diet studies in southeast Alaska showed high consumption of walleye pollock (SSFO 53.8%), herring (10.1%) and Pacific Hake (11.7%) from 2001-2004 (Tollit et al. 2015). In the Aleutian Islands and the Gulf of Alaska, Atka mackerel (*Pleurogrammus monopterygius*) and walleye pollock comprised the majority of Steller sea lion diets across multiple study periods, along with salmonids, rockfishes, and Pacific cod (Merrick et al. 1997, Sinclair and Zeppelin 2002, Sinclair et al. 2013).

Regional differences in Steller sea lion diet are partially due to differences in prey distribution. For example, higher prevalence of herring in the diets of Steller sea lions in southeastern Alaska and along coastal Washington has been correlated to the relative abundance of Pacific herring (Sinclair and Zeppelin 2002, Womble et al. 2005, Bredesen et al. 2006). American shad is a lesser known prey item for Steller sea lions, however, it has been previously documented in the Steller sea lion diet in coastal Washington (Scordino et al. 2022a). Increased diet proportion in this study is likely a result of the increasing American shad runs from the Columbia river (Hinrichsen et al. 2013).

Based on PERMANOVA, I found that season was the best predictor of individual diet composition, yet only explains 8.89% of the variation in diet. These results suggest that temporal variation influences Steller sea lion diet composition, but seasonality is not the sole explanation for diet differences. Drastic monthly changes in the proportion of prey species indicate that diet composition may be better explained at a finer temporal scale (Figure 7). For example, big skate was predominantly found in the month of May (24.1%), but was less present in the other spring months (March 0%, April 3.74%). These within season variations suggest that temporal trends in diet composition differences may best be explained by regional increases in prey availability.

Diet composition differences may be driven by seasonal prey availability, as prey species often undergo seasonal migration. For example, starry flounder was not found in Steller sea lion diet until May but comprised 35% of the overall diet in August (59.1% of the male diet, and 25.8% of the female diet). Starry flounder migrate seasonally, and are found nearshore during the spring and summer (Orcutt 1950, Morrow 1980, Ralston 2005). The seasonal nearshore distribution aligns with summer increases of starry flounder in Steller sea lion diet proportions found in this study, as well as in previous diet studies in the region (Scordino et al. 2022a). These

results further support the hypothesis that overall diet composition is likely influenced by local prey availability and abundance (Sigler et al. 2009, Womble et al. 2009). Diet composition differences may also be driven by size selectivity. Size selectivity of prey has been suggested by previous research in Alaska, which shows Steller sea lions predominantly consume fish between 14-42 cm (Tollit et al. 2015). Support for this hypothesis is shown in Appendix Table 1, where only 2 recovered lingcod (*Ophiodon elongatus*) otoliths were larger than 42 cm.

In comparison to previous hard parts studies of Steller sea lion diets along the coast of Washington, top prey species have shifted interannually. In 1989, 88% of scats contained Pacific hake, 24% contained spiny dogfish and 16% contained starry flounder (Scordino 2010). Top prey items determined from Steller sea lion scats 2010-2013 shifted towards higher prevalence of Clupeids, rockfishes, skates, dogfish, and salmon (Scordino et al. 2022a). Interannual shifts in prey distribution are likely the best explanation for shifts in top prey species consumed. For example, Pacific hake was a major component of Steller sea lion diet in 1989, yet only comprised 5.8% (SSFO) of the overall Steller sea diet during the 2010-2013 study period, and decreased to less than 1% (SSFO) of the sea lion diet determined in this study (Scordino 2010, Scordino et al. 2022a). Pacific hake distribution in recent years has shifted away from the Washington coast (Malick et al. 2020), which is likely driving the decreases in Pacific hake in Steller sea lion diet. Further, Pacific hake abundance depends heavily on strong year classes, which results in extreme annual variation (Malick et al. 2020).

Steller sea lion population diet diversity and relative individual specialization

Shannon diet diversity indices (determined from relative read abundance) of Steller sea lions along the northern Washington coast ranged from 2.03-2.59. These indices mirror the range

of Shannon diet diversity of Steller sea lion diets in 2010-2013, which range from 2.04-2.40 (Scordino et al. 2022a). These similarities in Shannon diversity index ranges indicate that diet diversity of this population has remained consistent interannually, despite shifts in top prey species.

I found the overall average proportional similarity index was 0.397 (95% CI = 0.023, R = 10,000). Male Steller sea lions (0.302 95% CI = 0.041) exhibited slightly more specialist tendencies than female Steller sea lions (0.347 95% CI = 0.028), although confidence levels overlapped across all months (Figure 9), suggesting that diet diversity was consistent across male and female individuals. Further, I found that relative individual specialization remained consistent within Steller sea lion diet across months (Figure 9).

Although numerous studies have focused on long term individual specialization, short term relative specialization is less described. Proportional similarity index has been previously used to describe short-term individual relative specialization in harbor seals in estuarine systems within the Salish Sea (Voelker et al. 2020). The average proportional similarity index found in this study was strikingly similar to the average value of relative individual diet variation of harbor seals (PS_i = 0.392, 95% CI = 0.013) (Voelker et al. 2020). In contrast to this study however, relative individual specialization in harbor seals was found to vary with both season and sex, with females showing higher levels of specialization across all seasons (Voelker et al. 2020). Harbor seals showed generalist trends with pelagic species, suggesting that pelagic foraging for prey may be common across pinniped species (Voelker et al. 2020). These results suggest that Steller sea lions along the coast of Washington exhibit a similar level of relative specialization to harbor seals in the Salish Sea, however, individual foraging habits may not be best described by sex and season.

Correlation analysis across all seasons and both sexes showed significant positive correlations between generalist diets and pelagic top species, including American shad, Pacific herring, walleye pollock, Chinook salmon and coho salmon (Figure 10). These correlations suggest that this population of Steller sea lions exhibiting short term generalist trends may be foraging for pelagic species. Further, Chinook salmon and coho salmon proportions show a strong correlation with each other (rho =0.64) as well as strong correlations with American shad (Chinook rho =0.41 and Coho rho =0.35). This suggests that foraging for salmonids and American shad occur in tandem and correlate to generalist dietary habits.

Steller sea lion diet: DNA metabarcoding and hard parts results

Top prey families detected by both DNA and hard parts analysis included clupeids, cods, rockfishes, and salmon. Top prey family proportions differed, as DNA metabarcoding detected a much higher proportion of clupeids (41.6%) than hard parts recovery (22.9%). For example, the overall relative read abundance of American shad was 16.7%, whereas split sample frequency of occurrence of American shad was 2.0%. These discrepancies are explained by the known bias that small prey items identified by fragile otoliths are underrepresented in hard parts recovery (Harvey et al. 2000, Sweeney and Harvey 2011). This study provides a useful comparison between diet proportions recovered by DNA metabarcoding and hard parts analysis of scat, novel to Steller sea lion diet studies. It is unsurprising that DNA and hard parts diet reconstruction did not fully align. Previous studies describing DNA metabarcoding suggest that differences in digestion of DNA in comparison to hard remains will effect results (Reed et al. 1997, Tollit et al. 2009, 2017, Thomas et al. 2017). This may be represented by the increase of cephalopod abundance in hard parts analysis, as cephalopod beaks may linger longer than other hard

remains, artificially inflating the proportion of cephalopods in diet (Deagle and Tollit 2007). Both DNA metabarcoding analysis and hard parts analysis show a large diversity in items consumed by Steller sea lions along the Washington coast. Further, both methods describe seasonal variation in prey consumed, consistent with previous findings within this region (Akmajian et al. 2017, Scordino et al. 2022a) and across Steller sea lion populations throughout the Pacific Ocean (Sinclair and Zeppelin 2002, Riemer et al. 2011).

Influence of Steller sea lion sex on diet composition

I determined the sex of 159 female and 79 male scats of Steller sea lion scats using custom designed qPCR Taqman assays. Overall, 89.9% of scats collected where successfully identified to predator sex. The observed sex ratios from demographic counts correlated highly with sex ratios found via qPCR analysis of scat samples (Figure 5). The success of this correlation may reflect and corroborate the assumption that scat sampling of marine predator populations is representative of the population sampled.

This study successfully characterized diet composition of male and female Steller sea lions, however variation in diet composition was not explained by sex. During the winter and spring, the number of male sea lion scats analyzed (winter n=23, spring n= 20) was much lower in comparison to the number of female scats collected (winter n=68, spring n = 56). Prior studies suggest that at least 59 samples are necessary to detect all potential prey items, whereas over 90 scat samples are necessary to detect spatial or temporal changes (Trites and Joy 2005). Thus, scat sampling of Steller sea lions with the goal of investigating sex specific effects may require increased sampling effort. This study also documented monthly fluctuations of Steller sea lion sex ratios with both scat analysis and observational counts during the study period. These

changes in sex ratio show increases in male Steller sea lions during the summer of 2022, suggesting that changes in diet in summer months may be influenced by changes in sex ratios.

The results of this study differ from previous studies that suggest that there are sex-specific differences in the diet of wild Steller sea lion populations (Trites and Calkins 2008). Trites and Calkins found that both sex and age influenced the diet of Steller sea lions. Thus, sex-specific diet differences may be unapparent as my data do not describe the age of predator in relation to their diet. For example, juvenile male individuals consume a higher percentage of body mass per day than adult males, and in turn, may exhibit differing dietary preferences from adults (Winship et al. 2006). Currently, there are no methods for distinguishing between scats deposited by adult and juvenile male sea lions, and thus these dietary differences due to predator age cannot be differentiated.

Study limitations and biases

It is critical to note limitations to studies extrapolating diet proportions based on either DNA metabarcoding or hard parts data. Both analyses are subject to prey-item specific digestion bias, which results in different dietary proportions determined than the actual ingested prey proportions (Lance et al. 2001, Bowen and Iverson 2013, Thomas et al. 2014, 2016). Digestion biases in hard parts have been well documented in marine mammal diets, and thus the biases for specific prey items are well known (Bowen and Iverson 2013). Biases in DNA metabarcoding must be calibrated for both predator species and expected prey species (Thomas et al. 2016). One potential bias may occur if prey species within the same family have highly conserved target regions across multiple species within one family, as seen in salmonids. In this study, DNA metabarcoding was unable to differentiate most rockfish prey items to a species level, indicating

that the 16s region was highly conserved in Sebastidae, and suggesting that a more specific probe would be necessary to differentiate at a species level. However, when used to estimate population level diet estimates, relative read abundances of DNA have been shown to reflect relative biomass consumed (Deagle et al. 2019, Thomas et al. 2022) and thus RRA can be compared within a study. It is critical to note that direct comparisons of diet composition between methods will likely produce different results due to these biases, especially within individual scats.

This study also relied on certain assumptions for data analysis that may bias results. First, the bioinformatics portion of DNA metabarcoding analysis relies on multiple methods to remove potential environmental contamination or secondary prey items from sample analysis. For example, it is common practice to remove species <1% of read abundance within a sample, which can in turn reduce the species richness within diet (Littleford-Colquhoun et al. 2022). However, the impacts of threshold reduction of diet diversity are minimal, and thus thresholding methods used in this study are unlikely to bias overall results. (Littleford-Colquhoun et al. 2022). Second, the proportional similarity indices (PS_i) used to represent relative individual specialization were measured using a cross-sectional approach, meaning that temporal changes in diet are not tracked on an individual basis (Araújo et al. 2011, Voelker et al. 2020). Thus, true specialization cannot be quantified and is likely representative of short-term prey decisions. However, analysis of proportional similarity indices via the cross-sectional approach of scat collection allows for the exploration of short term individual specialization, which can be used to interpret overall foraging behavior of a population (Voelker et al. 2020). Further, the PS_i metric is also influenced by sample size, which is skewed towards female samples in this study. Bias in hard parts data analysis occurs since SSFO assumes that all prey items are consumed at the same

proportion. Therefore, this calculation overestimates the presence of small and/or rare prey items and underestimates the presence of large prey (Olesiuk et al. 1990). To mitigate the influence of this bias, I relied on relative read abundance of DNA metabarcoding to perform my analyses related to diet consumption. However, the hard parts identification strengthened the diet reconstruction by providing classification of age related structures (Bowen and Iverson 2013, Thomas et al. 2017).

Conclusion

This study documents the first published usage of DNA metabarcoding analysis on Steller sea lion diets and compared this method with traditional hard parts analysis. The predominant prey items of Steller sea lions in this study were clupeids, Pacific herring and American shad, showing interannual variation from previous diet studies in this region. I found that diet composition in Steller sea lions was explained in part by season and I hypothesize that seasonal variation occurs due to changing prey abundance along the coast of Washington State. Proportional similarity indices show that monthly populations of Steller sea lions exhibit some level of short term, relative specialization, but do not show specialization of specific prey types. However, individuals exhibiting generalist foraging techniques showed correlation with pelagic prey items such as American shad, Pacific herring, walleye pollock and Pacific salmon. In summary, Steller sea lions exhibiting generalist pelagic foraging strategies consume species of conservation concern such as Pacific herring and Pacific salmon, indicating that continued monitoring and research of this region combined with biomass modeling is necessary to determine predation impact on these prey species.

REFERENCES

Adams, J., I. C. Kaplan, B. Chasco, K. N. Marshall, A. Acevedo-Gutiérrez, and E. J. Ward. 2016. A century of Chinook salmon consumption by marine mammal predators in the Northeast Pacific Ocean. Ecological Informatics 34:44–51.

Akmajian, A. M., J. J. Scordino, and A. Acevedo-Gutiérrez. 2017. Year-round algal toxin exposure in free-ranging sea lions. Marine Ecology Progress Series 583:243–248.

Allen, M. J., and G. B. Smith. 1988. Atlas and zoogeography of common fishes in the Bering Sea and Northeastern Pacific. NOAA Technical Report NMFS 66.

Allyn, E. M., and J. J. Scordino. 2020. Entanglement rates and haulout abundance trends of Steller (*Eumetopias jubatus*) and California (*Zalophus californianus*) sea lions on the north coast of Washington state. PLoS ONE 15:1–21.

Anderson, M. J., and D. C. I. Walsh. 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? Ecological Monographs 83:557–574.

Atlas, W. I., N. C. Ban, J. W. Moore, A. M. Tuohy, S. Greening, A. J. Reid, N. Morven, E. White, W. G. Housty, J. A. Housty, C. N. Service, L. Greba, S. Harrison, C. Sharpe, K. I. R. Butts, W. M. Shepert, E. Sweeney-Bergen, D. MacIntyre, M. R. Sloat, and K. Connors. 2021. Indigenous Systems of Management for Culturally and Ecologically Resilient Pacific Salmon (*Oncorhynchus* spp.) Fisheries. BioScience 71:186–204.

Beamish, R. J., and C. Mahnken. 2001. A critical size and period hypothesis to explain natural regulation of salmon abundance and the linkage to climate and climate change. Progress in Oceanography 49:423–437.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the False Discovery Rate : A Practical and Powerful Approach to Multiple Testing.cJournal of the Royal Statistical Society. 57:289–300.

Berry, T. E., S. K. Osterrieder, D. C. Murray, M. L. Coghlan, A. J. Richardson, A. K. Grealy, M. Stat, L. Bejder, and M. Bunce. 2017. DNA metabarcoding for diet analysis and biodiversity: A case study using the endangered Australian sea lion (*Neophoca cinerea*). Ecology and Evolution 7:5435–5453.

Bi, H., R. E. Ruppel, W. T. Peterson, and E. Casillas. 2008. Spatial distribution of ocean habitat of yearling Chinook (*Oncorhynchus tshawytscha*) and coho (*Oncorhynchus kisutch*) salmon off Washington and Oregon, USA. Fisheries Oceanography 17:463–476.

Bigg, M. A. 1985. Status of the Steller Sea Lion (*Eumetopias jubatus*) and California Sea Lion (*Zalophus californianus*) in British Columbia. Canadian Special Publication of Fisheries and Aquatic Sciences.

Bolnick, D. I., R. Svanbäck, J. A. Fordyce, L. H. Yang, J. M. Davis, C. D. Hulsey, and M. L. Forister. 2003. The ecology of individuals: Incidence and implications of individual specialization. American Naturalist 161:1–28.

Bolnick, D. I., L. H. Yang, J. A. Fordyce, J. M. Davis, and R. Svanbäck. 2002. Measuring individual-level resource specialization. Ecology 83:2936–2941.

Bolyen, E., J. R. Rideout, M. R. Dillon, N. A. Bokulich, C. C. Abnet, G. A. Al-Ghalith, H. Alexander, E. J. Alm, M. Arumugam, F. Asnicar, Y. Bai, J. E. Bisanz, K. Bittinger, A. Brejnrod, C. J. Brislawn, C. T. Brown, B. J. Callahan, A. M. Caraballo-Rodríguez, J. Chase, E. K. Cope, R. Da Silva, C. Diener, P. C. Dorrestein, G. M. Douglas, D. M. Durall, C. Duvallet, C. F. Edwardson, M. Ernst, M. Estaki, J. Fouquier, J. M. Gauglitz, S. M. Gibbons, D. L. Gibson, A. Gonzalez, K. Gorlick, J. Guo, B. Hillmann, S. Holmes, H. Holste, C. Huttenhower, G. A. Huttley, S. Janssen, A. K. Jarmusch, L. Jiang, B. D. Kaehler, K. Bin Kang, C. R. Keefe, P. Keim, S. T. Kelley, D. Knights, I. Koester, T. Kosciolek, J. Kreps, M. G. I. Langille, J. Lee, R. Ley, Y. X. Liu, E. Loftfield, C. Lozupone, M. Maher, C. Marotz, B. D. Martin, D. McDonald, L. J. McIver, A. V. Melnik, J. L. Metcalf, S. C. Morgan, J. T. Morton, A. T. Naimey, J. A. Navas-Molina, L. F. Nothias, S. B. Orchanian, T. Pearson, S. L. Peoples, D. Petras, M. L. Preuss, E. Pruesse, L. B. Rasmussen, A. Rivers, M. S. Robeson, P. Rosenthal, N. Segata, M. Shaffer, A. Shiffer, R. Sinha, S. J. Song, J. R. Spear, A. D. Swafford, L. R. Thompson, P. J. Torres, P. Trinh, A. Tripathi, P. J. Turnbaugh, S. Ul-Hasan, J. J. J. van der Hooft, F. Vargas, Y. Vázquez-Baeza, E. Vogtmann, M. von Hippel, W. Walters, Y. Wan, M. Wang, J. Warren, K. C. Weber, C. H. D. Williamson, A. D. Willis, Z. Z. Xu, J. R. Zaneveld, Y. Zhang, Q. Zhu, R. Knight, and J. G. Caporaso. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology 37:852–857.

Bowen, W. D., and S. J. Iverson. 2013. Methods of estimating marine mammal diets: A review of validation experiments and sources of bias and uncertainty. Marine Mammal Science 29:719–754.

Braje, T. J., and T. C. Rick. 2011. Human Impacts on Seals, Sea Lions and Sea Otters: Integrating Archaeology and Ecology in the Northeast Pacific. University of Califonia Press.

Bredesen, E. L., A. P. Coombs, and A. W. Trites. 2006. Relationship between Steller Sea Lion Diets and Fish Distributions in the Eastern North Pacific. Pages 131–139 *in* A. W. Trites, S. K. Atkinson, D. P. DeMaster, T. S. Gelatt, L. W. Fritz, L. D. Rea, and K. M. Wynne, editors. Sea Lions of the World.

Butler, V. L., and S. K. Campbell. 2004. Resource intensification and resource depression in the Pacific Northwest of North America: A zooarchaeological review. Journal of World Prehistory 18:327–405.

Cammen, K. M., D. B. Rasher, and R. S. Steneck. 2019. Predator recovery, shifting baselines, and the adaptive management challenges they create. Ecosphere 10(2). DOI 10.1002/ecs2.2579

Carretta, J. V., K. A. Forney, E. M. Oleson, D. W. Weller, A. R. Lang, J. Baker, M. M. Muto, B. Hanson, A. J. Orr, H. R. Huber, M. S. Lowry, J. Barlow, J. E. Moore, D. Lynch, L. Carswell, and R. L. Brownell. 2018. 2018. U.S. Pacific Marine Mammal Stock Assessments: 2017. US Department of Commerce. NOAA Technical Memorandum NMFS-SWFSC-602. Online access: https://doi.org/10.7289/V5/TM-SWFSC-602

Carretta, J. V., E. M. Oleson, D. W. Weller, A. R. Lang, K. A. Forney, J. Baker, B. Hanson, K. Martien, M. M. Muto, A. J. Orr, H. R. Huber, M. S. Lowry, J. Barlow, D. Lynch, L. Carswell, R. L. Brownell, and D. K. Mattila. 2013. Pacific Marine Mammal Stock Assessments: 2012. U.S. Department of Commerce, NOAA Technical Memorandum, NMFS-SWFSC-504.

Cederholm, C. J., D. H. Johnson, R. E. Bilby, L.G. Dominguez, A. M. Garrett, W. H. Graeber, E. L. Greda, M. D. Kunze, B.G. Marcot, J. F. Palmisano, R. W. Plotnikoff, W. G. Pearcy, C. A. Simenstad, and P. C. Trotter. 2000. Pacific Salmon and Wildlife - Ecological Contexts, Relationships, and Implications for Management. Special Edition Technical Report.

Chasco, B., I. C. Kaplan, A. C. Thomas, A. Acevedo-Gutiérrez, D. P. Noren, M. J. Ford, M. B. Hanson, J. J. Scordino, S. J. Jeffries, K. N. Marshall, A. O. Shelton, C. Matkin, B. J. Burke, and E. J. Ward. 2017a. Competing tradeoffs between increasing marine mammal predation and fisheries harvest of Chinook salmon. Scientific Reports 7:1–14.

Chasco, B., I. C. Kaplan, A. C. Thomas, A. Acevedo-Gutiérrez, D. P. Noren, M. J. Ford, M. B. Hanson, J. J. Scordino, S. J. Jeffries, S. F. Pearson, K. N. Marshall, and E. J. Ward. 2017b. Estimates of chinook salmon consumption in Washington State inland waters by four marine mammal predators from 1970 to 2015. Canadian Journal of Fisheries and Aquatic Sciences 74:1173–1194.

Chittenden, C. M., R. M. Sweeting, C. M. Neville, K. Young, M. Galbraith, E. Carmack, S. Vagle, M. Dempsey, J. Eert, and R. J. Beamish. 2018. Estuarine and marine diets of out-migrating Chinook salmon smolts in relation to local zooplankton populations, including harmful blooms. Estuarine, Coastal and Shelf Science 200:335–348.

Codron, J., D. Codron, M. Sponheimer, K. Kirkman, K. J. Duffy, E. J. Raubenheimer, J. L. Mélice, R. Grant, M. Clauss, and J. A. Lee-Thorp. 2012. Stable isotope series from elephant ivory reveal lifetime histories of a true dietary generalist. Proceedings of the Royal Society B: Biological Sciences 279:2433–2441.

Coté, C. 2010. Spirits of Our Whaling Ancestors: Revitalizing Makah and Nuu-chah-nulth Traditions. 1st edition. University of Washington Press, Seattle.

Cottrell, P. E., and A. W. Trites. 2002. Classifying prey hard part structures recovered from fecal remains of captive Steller sea lions (*Eumetopias jubatus*). Marine Mammal Science 18:525–539.

D'Agnese, E., D. Lambourn, J. Rice, D. Duffield, J. Huggins, T. Spraker, S. Raverty, T. Kuzmina, M. E. Grigg, K. Wilkinson, S. J. Jeffries, and W. Smith. 2020. Reemergence of Guadalupe fur seals in the U.S. Pacific Northwest: The epidemiology of stranding events during 2005–2016. Marine Mammal Science 36:828–845.

Davis, K. A., N. S. Banas, S. N. Giddings, S. A. Siedlecki, P. MacCready, E. J. Lessard, R. M. Kudela, and B. M. Hickey. 2014. Estuary-enchanced upwelling of marine nutrients fuels coastal productivity in the U.S. Pacific Northwest. Journal of Geophysical Research: Oceans 119:8778–8799.

Deagle, B. E., R. Kirkwood, and S. N. Jarman. 2009. Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. Molecular Ecology 18:2022–2038.

Deagle, B. E., A. C. Thomas, J. C. McInnes, L. J. Clarke, E. J. Vesterinen, E. L. Clare, T. R. Kartzinel, and J. P. Eveson. 2019. Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? Molecular Ecology 28:391–406.

Deagle, B. E., A. C. Thomas, A. K. Shaffer, A. W. Trites, and S. N. Jarman. 2013. Quantifying sequence proportions in a DNA-based diet study using Ion Torrent amplicon sequencing: Which counts count? Molecular Ecology Resources 13:620–633.

Deagle, B. E., and D. J. Tollit. 2007. Quantitative analysis of prey DNA in pinniped faeces: Potential to estimate diet composition? Conservation Genetics 8:743–747.

Deagle, B. E., D. J. Tollit, S. N. Jarman, M. A. Hindell, A. W. Trites, and N. J. Gales. 2005. Molecular scatology as a tool to study diet: Analysis of prey DNA in scats from captive Steller sea lions. Molecular Ecology 14:1831–1842.

Drake, L. E., J. P. Cuff, R. E. Young, A. Marchbank, E. A. Chadwick, and W. O. C. Symondson. 2022. An assessment of minimum sequence copy thresholds for identifying and reducing the prevalence of artefacts in dietary metabarcoding data. Methods in Ecology and Evolution 13:694–710.

Ellingsen, K. E., N. G. Yoccoz, T. Tveraa, K. T. Frank, E. Johannesen, M. J. Anderson, A. V. Dolgov, and N. L. Shackell. 2020. The rise of a marine generalist predator and the fall of beta diversity. Global Change Biology 26:2897–2907.

Essington, T. E. 2004. Getting the right answer from the wrong model: Evaluating the sensitivity of multispecies fisheries advice to uncertain species interactions. Bulletin of Marine Science 74:563–581.

Etnier, M. A. 2002. Occurrences of guadalupe fur seals (*Arctocephalus townsendi*) on the Washington coast over the past 500 years. Marine Mammal Science 18:551–557.

Etnier, M. A. 2007. Defining and identifying sustainable harvests of resources: Archaeological examples of pinniped harvests in the eastern North Pacific. Journal for Nature Conservation 15:196–207.

Etnier, M. A., and J. Sepez. 2008. Chapter 9: Changing Patterns of Sea Mammal Exploitation Among the Makah. *in* Time and Change: Archaeological and Anthropological Perspecitves on the Long-Term in Hunter-Gatherer Societies.

Fisher, J. P., and W. G. Pearcy. 1988. Growth of Juvenile Coho Salmon (*Oncorhynchus kisutch*) off Oregon and Washington, USA, in years of different coastal upwelling. Canadian Journal of Fisheries and Aquatic Sciences 45:1036–1044.

Fritz, L., B. Brost, E. Laman, K. Luxa, K. Sweeney, J. Thomason, D. Tollit, W. Walker, and T. Zeppelin. 2019. A re-examination of the relationship between steller sea lion (*Eumetopias jubatus*) diet and population trend using data from the Aleutian islands. Canadian Journal of Zoology 97:1137–1155.

Gudmundson, C. J., T.K. Zeppelin, and R. R. Ream. 2000. Application of two methods for determining diet of northern fur seals (*Callorhinus ursinus*). Fisheries Bulletin 104:445–455.

Hardy, N., T. Berry, B. P. Kelaher, S. D. Goldsworthy, M. Bunce, M. A. Coleman, B. M. Gillanders, S. D. Connell, M. Blewitt, and W. Figueira. 2017. Assessing the trophic ecology of top predators across a recolonisation frontier using DNA metabarcoding of diets. Marine Ecology Progress Series 573:237–254.

Harvey, J. T., T. R. Loughlin, M. a Perez, and D. S. Oxman. 2000. Relationship between Fish Size and Otolith Length for 63 Species of Fishes from the Eastern North Pacific Ocean. NOAA Technical Report NMFS 150.

Hayden, B., C. Harrod, and K. K. Kahilainen. 2014. Dual fuels: Intra-annual variation in the relative importance of benthic and pelagic resources to maintenance, growth and reproduction in a generalist salmonid fish. Journal of Animal Ecology 83:1501–1512.

Healey, M. C. 1991. Life history of Chinook Salmon (*Oncorhynchus tshawytscha*), Pacific Salmon Life Histories. Vancouver, British Columbia: UBC Press. 313-393

Hebert, P. D. N., A. Cywinska, S. L. Ball, and J. R. DeWaard. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences 270:313–321.

Hinrichsen, R. A., D. J. Hasselman, C. C. Ebbesmeyer, and B. A. Shields. 2013. The role of impoundments, temperature, and discharge on colonization of the Columbia River Basin,

USA, by nonindigenous American shad. Transactions of the American Fisheries Society 142:887–900.

Hjort, J. 1914. Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. Rapport Process-Verbaux Réunions Conseil Perm. Intern. Exploration Mer 20:1–228.

Höss, M., M. Kohn, S. Pääbo, F. Knauer, and W. Schröder. 1992. Excrement analysis by PCR. Nature 359:199.

Howard, S. M. S., M. M. Lance, S. J. Jeffries, and A. Acevedo-Gutiérrez. 2013. Fish consumption by harbor seals (*Phoca vitulina*) in the San Juan Islands, Washington. Fishery Bulletin 111:27–41.

Jarman, S. N., B. E. Deagle, and N. J. Gales. 2004. Group-specific polymerase chain reaction for DNA-based analysis of species diversity and identity in dietary samples. Molecular Ecology 13:1313–1322.

Jeanniard-Du-Dot, T., A. C. Thomas, Y. Cherel, A. W. Trites, and C. Guinet. 2017. Combining hard-part and DNA analyses of scats with biologging and stable isotopes can reveal different diet compositions and feeding strategies within a fur seal population. Marine Ecology Progress Series 584:1–16.

Jeffries, S., H. R. Huber, J. Calambokidis, and J. L. Laake. 2003. Trends and Status of Harbor Seals in Washington State: 1978-1999. Journal of Wildlife Management 67:208–219.

Johnson, K. F., A. M. Edwards, A. M. Berger, and C. J. Grandin. 2021. Status of the Pacific Hake (whiting) stock in U.S. and Canadian waters in 2021.

Jones, R., C. Rigg, and L. Lee. 2010. Haida Marine Planning: First Nations as a Partner in Marine Conservation. Ecology and Society 15:12.

Kassambara, A. 2021. 'rstatix': Pipe-Friendly Framework for Basic Statistical tests. R package version 0.7.0, Online access: https://CRAN.R-project.org/package=rstatix.

Kastelein, R. A., N. Vaughan, and P. R. Wiepkema. 1990. The food consumption of Steller sea lions *(Eumetopias jubatus*). Aquatic Mammals 15:137–144.

Kernaléguen, L., N. Dorville, D. Ierodiaconou, A. J. Hoskins, A. M. M. Baylis, M. A. Hindell, J. Semmens, K. Abernathy, G. J. Marshall, Y. Cherel, and J. P. Y. Arnould. 2016. From video recordings to whisker stable isotopes: a critical evaluation of timescale in assessing individual foraging specialisation in Australian fur seals. Oecologia 180:657–670.

Kvitrud, M. A., S. D. Riemer, R. F. Brown, M. R. Bellinger, and M. A. Banks. 2005. Pacific harbor seals (Phoca vitulina) and salmon: Genetics presents hard numbers for elucidating predator-prey dynamics. Marine Biology 147:1459–1466.

Laake, J. L., P. Browne, R. L. Delong, and H. R. Huber. 2002. Pinniped diet composition: a comparison of estimation models. Page Fish. Bull.

Lance, M. M., W. Y. Chang, S. J. Jeffries, S. F. Pearson, and A. Acevedo-Gutiérrez. 2012. Harbor seal diet in northern Puget Sound: Implications for the recovery of depressed fish stocks. Marine Ecology Progress Series 464:257–271.

Lance, M. M., A. J. Orr, S. D. Riemer, M. J. Weise, and J. L. Laake. 2001. Pinniped Food Habits and Prey Identification Techniques Protocol.

Littleford-Colquhoun, B. L., P. T. Freeman, V. I. Sackett, C. V. Tulloss, L. M. McGarvey, C. Geremia, and T. R. Kartzinel. 2022. The precautionary principle and dietary DNA metabarcoding: Commonly used abundance thresholds change ecological interpretation. Molecular ecology 31:1615–1626.

Losee, J. P., N. W. Kendall, and A. Dufault. 2019. Changing salmon: An analysis of body mass, abundance, survival, and productivity trends across 45 years in Puget Sound. Fish and Fisheries 20:934–951.

Luxa, K., and A. Acevedo-Gutiérrez. 2013. Food habits of harbor seals (*Phoca vitulina*) in two estuaries in the central Salish Sea. Aquatic Mammals 39:10–22.

MacFadyen, A., B. M. Hickey, and W. P. Cochlan. 2008. Influences of the Juan de Fuca Eddy on circulation, nutrients, and phytoplankton production in the northern California Current System. Journal of Geophysical Research: Oceans 113:1–19.

MacFadyen, A., B. M. Hickey, and M. G. G. Foreman. 2005. Transport of surface waters from the Juan de Fuca eddy region to the Washington coast. Continental Shelf Research 25:2008–2021.

Makah Tribe. 2020. State of Our Watersheds Report Northwest Olympic Peninsula. Northwest Treaty Tribes, Northwest Indian Fisheries Commission. Online access: https://nwifc.org/publications/state-of-our-watersheds/

Malick, M. J., S. A. Siedlecki, E. L. Norton, I. C. Kaplan, M. A. Haltuch, M. E. Hunsicker, S. L. Parker-Stetter, K. N. Marshall, A. M. Berger, A. J. Hermann, N. A. Bond, and S. Gauthier. 2020. Environmentally Driven Seasonal Forecasts of Pacific Hake Distribution. Frontiers in Marine Science 7:1–12.

Manishin, K. A., C. J. Cunningham, P. A. H. Westley, and A. C. Seitz. 2021. Can late stage marine mortality explain observed shifts in age structure of Chinook salmon? PLoS ONE 16:1–14.

Martins, E. G., M. S. Araújo, V. Bonato, and S. F. Dos Reis. 2008. Sex and season affect individual-level diet variation in the neotropical marsupial *Gracilinanus microtarsus* (Didelphidae). Biotropica 40:132–135.

Matejusová, I., F. Bland, A. J. Hall, R. N. Harris, M. Snow, A. Douglas, and S. J. Middlemas. 2013. Real-time PCR assays for the identification of harbor and gray seal species and sex: A molecular tool for ecology and management. Marine Mammal Science 29:186–194.

McCue, L. M., C. C. Fahy, J. Greenman, and K. Wilkinson. 2021. Endangered Species Act Status Review Report for the Guadalupe Fur Seal (*Arctocephalus townsendi*). Long Beach, CA.

McHuron, E. A., P. W. Robinson, S. E. Simmons, C. E. Kuhn, M. Fowler, and D. P. Costa. 2016. Foraging strategies of a generalist marine predator inhabiting a dynamic environment. Oecologia 182:995–1005.

McKnight, D. T., R. Huerlimann, D. S. Bower, L. Schwarzkopf, R. A. Alford, and K. R. Zenger. 2019. microDecon: A highly accurate read-subtraction tool for the post-sequencing removal of contamination in metabarcoding studies. Environmental DNA 1:14–25.

Merrick, R. L., M. K. Chumbley, and G. V. Byrd. 1997. Diet diversity of Steller sea lions (*Eumetopias jubatus*) and their population decline in Alaska: a potential relationship.

Merrick, R. L., and T. R. Loughlin. 1997. Foraging behavior of adult female and young-of-the-year Steller sea lions in Alaskan waters. Canadian Journal of Zoology:776–786.

Morrow, J. E. 1980. The freshwater fishes of Alaska. Alaska Northwest Publishing Company, Anchorage, Alaska.

Moss, M. L., and R. J. Losey. 2011. Native American use of seals, sea lions, and sea otters in estuaries of Northern Oregon and Southern Washington. Human Impacts on Seals, Sea Lions, and Sea Otters: Integrating Archaeology and Ecology in the Northeast Pacific:167–195.

Muto, M. M., V. T. Helker, B. J. Delean, R. P. Angliss, P. L. Boveng, J. M. Breiwick, B. M. Brost, M. F. Cameron, P. J. Clapham, S. P. Dahle, M. E. Dahlheim, B. S. Fadely, M. C. Ferguson, L. W. Fritz, R. C. Hobbs, Y. V. Ivashchenko, A. S. Kennedy, J. M. London, S. A. Mizroch, R. R. Ream, E. L. Richmond, K. E. W. Shelden, K. L. Sweeney, R. G. Towell, P. R. Wade, J. M. Waite, and A. N. Zerbini. 2020. CITATION CHECK: Steller Sea Lion (*Eumetopias jubatus*) Eastern U.S. Stock 2019. NOAA Technical Memorandum AFSC-404 551:18–29.

Nelson, B. W., V. Christensen, and C. J. Walters. 2020. Rebuilding the Georgia Strait sport fishery through marine mammal culling. University of British Columbia, Institute for the Oceans and Fisheries.

Nelson, B. W., S. F. Pearson, J. H. Anderson, S. J. Jeffries, A. C. Thomas, W. A. Walker, A. Acevedo-Gutiérrez, I. M. Kemp, M. M. Lance, A. Louden, and M. R. Voelker. 2021. Variation in predator diet and prey size affects perceived impacts to salmon species of high conservation concern. Canadian Journal of Fisheries and Aquatic Sciences 78:1661–1676.

Newby, T. C. 1973. Changes in the Washington State Harbor Seal Population, 1942-1972. The Murrelet 54:4–6.

Nifong, J. C., C. A. Layman, and B. R. Silliman. 2015. Size, sex and individual-level behaviour drive intrapopulation variation in cross-ecosystem foraging of a top-predator. Journal of Animal Ecology 84:35–48.

Oksanen, J., G. L. Simpson, F. G. Blanchet, R. Kindt, Pierre Legendre, P. R. Minchin, R. B. O'Hara, P. Solymos, M. H. H. Stevens, E. Szoecs, H. Wagner, M. Barbour, M. Bedward, B. Bolker, D. Borcard, G. Carvalho, M. Chirico, M. De Caceres, S. Durand, H. B. A. Evangelista, R. FitzJohn, M. Friendly, B. Furneaux, G. Hannigan, M. O. Hill, L. Lahti, D. McGlinn, M.-H. Ouellette, E. R. Cunha, T. Smith, A. Stier, C. J. F. Ter Braak, and J. Weedon. 2022. _vegan: Community Ecology Package_. R package version 2.6-2, https://CRAN.R-project.org/package=vegan.

Olesiuk, P. F., M. A. Bigg, G. M. Ellis, S. J. Crockford, and R. J. Wigen. 1990. An Assessment of the Feeding Habits of Harbour Seals (*Phoca vitulina*) in the Strait of Georgia, British Columbia, Based on Scat Analysis. Can. Tech. Rep. Fish. Aquat. Sci. 1730: 135 p.

Oleson, E. M., J. Calambokidis, E. Falcone, G. S. Schorr, and J. A. Hildebrand. 2009. FY07 Grant Report: Acoustic and Visual Monitoring for Cetaceans along the Outer Washington Coast.

Olivier, P. A., R. Andrews, V. Burkanov, and R. W. Davis. 2022. Diving behavior, foraging strategies, and energetics of female Steller sea lions during early lactation. Journal of Experimental Marine Biology and Ecology 550:151707.

Orcutt, H. G. 1950. The Life History of the Starry Flounder *Platichthys stellatus* (Pallas). Fish Bulletin:1–64.

Orr, A. J., A. S. Banks, S. Mellman, H. R. Huber, R. L. Delong, and R. F. Brown. 2004. Examination of the foraging habits of Pacific harbor seal (*Phoca vitulina richardsi*) to describe their use of the Umpqua River, Oregon, and their predation on salmonids. Fishery Bulletin 102:108–117.

Orr, A. J., J. L. Laake, M. I. Dhruv, A. S. Banks, R. L. Delong, and H. R. Huber. 2003. Comparison of Processing Pinniped Scat Samples Using a Washing Machine and Nested Sieves. Source: Wildlife Society Bulletin.

Palsson, W. A., T. Tsou, G. G. Bargmann, R. M. Buckley, J. E. West, M. Lou Mills, Y. W. Cheng, and R. E. Pacunski. 2009. CITE CHECK: The Biology and Assessment of Rockfishes in Puget Sound. Washington Department of Fish and Wildlife:208.

Pearcy, W. G., and J. P. Fisher. 1990. Distribution and Abundance of Juvenile Salmonids off Oregon. Fisheries (Bethesda):1981–1985.

Petersen, J. R., J. J. Scordino, C. I. Svec, R. H. Buttram, M. R. Gonzalez, and J. Scordino. 2020. Use of the traditional halibut hook of the Makah Tribe, the čibu·d, reduces bycatch in recreational halibut fisheries. PeerJ 2020:1–16.

Pitcher, K. W., P. F. Olesiuk, R. F. Brown, M. S. Lowry, S. J. Jeffries, J. L. Sease, W. L. Perryman, C. E. Stinchcomb, L. F. Lowry, and Email. 2007. Abundance and distribution of the eastern North Pacific Steller sea lion (*Eumetopias jubatus*) population. Fisheries Bulletin:102–115.

Purcell, M., G. Mackey, E. LaHood, H. R. Huber, and L. K. Park. 2004. Molecular methods for the genetic identification of salmonid prey from Pacific harbor seal (*Phoca vitulina richardsi*) scat. Fishery Bulletin 102:213–220.

Quevedo, M., R. Svanbäck, and P. Eklöav. 2009. Intrapopulation niche partitioning in a generalist predator limits food web connectivity. Ecology 90:2263–2274.

Ralston, S. 2005. An Assessment of Starry flounder off California, Oregon, and Washington. NOAA Fisheries Stock Assessment.

Reed, J. Z., D. J. Tollit, P. M. Thompson, and W. Amos. 1997. Molecular scatology: The use of molecular genetic analysis to assign species, sex and individual identity to seal faeces. Molecular Ecology 6:225–234.

Riddell, B. E., R. D. Brodeur, A. V Bugaev, P. Moran, J. M. Murphy, J. A. Orsi, M. Trudel, L. A. Weitkamp, B. K. Wells, and A. C. Wertheimer. 2018. Ocean Ecology of Chinook Salmon. Pages 555–696 *in* R. J. Beamish, editor. The Ocean Ecology of Pacific Salmon and Trout.

Riemer, S. D., B. E. Wright, and R. F. Brown. 2011. Food habits of Steller sea lions (*Eumetopias jubatus*) off Oregon and northern California, 1986-2007.

Rita, D., M. Drago, F. Galimberti, and L. Cardona. 2017. Temporal consistency of individual trophic specialization in southern elephant seals Mirounga leonina. Marine Ecology Progress Series 585:229–242.

Rosalino, L. M., F. Loureiro, D. W. Macdonald, and M. Santon-Reis. 2005. Dietary shifts of the badger (*Meles meles*) in Mediterranean woodlands: An opportunistic forager with seasonal specialisms. Mammalian Biology 70:12–23.

Rothstein, A. P. 2015. Non-invasive genetic tracking of Harbor Seals (*Phoca vitulina*). Unpublished Master's Thesis. Western Washington University.

Scheffer, T. H. 1928. Precarious Status of the Seal and Sea-Lion on Our Northwest Coast. Journal of Mammalogy 9:10-16.

Schwarz, D., S. M. Spitzer, A. C. Thomas, C. M. Kohnert, T. R. Keates, and A. Acevedo-Gutiérrez. 2018. Large-scale molecular diet analysis in a generalist marine mammal reveals male preference for prey of conservation concern. Ecology and Evolution 8:9889–9905.

Schweigert, J. F., J. L. Boldt, L. Flostrand, and J. S. Cleary. 2010. A review of factors limiting recovery of Pacific herring stocks in Canada. ICES Journal of Marine Science 67:1903–1913.

Scordino, J. 2010. West Coast Pinniped Program Investigations on California Sea Lion and Pacific Harbor Seal Impacts on Salmonids and Other Fishery Resources Pacific States Marine Fisheries Commission Executive Summary.

Scordino, J. J., A. M. Akmajian, and S. L. Edmondson. 2022a. Dietary niche overlap and prey consumption for the Steller sea lion (*Eumetopias jubatus*) and California sea lion (*Zalophus californianus*) in northwest Washington during 2010–2013. Fishery Bulletin 120:39–54.

Scordino, J. J., A. M. Akmajian, and S. D. Riemer. 2013. Research and Education/Outreach to Benefit ESA Listed and Recently Delisted Marine Mammals of Northwest Washington.

Scordino, J. J., C. Marshall, A. M. Akmajian, D. Shay, and R. James. 2022b. Consumption of Pacific salmon (*Oncorhynchus* spp.) by California sea lions (*Zalophus californianus*) and Steller sea lions (*Eumetopias jubatus*) in northwest Washington during 2010–2013. Fishery Bulletin:150–161.

Sepez, J. 2008. Historical ecology of Makah subsistence foraging patterns. Journal of Ethnobiology 28:110–133.

Shelton, A. O., W. Satterthwaite, E. J. Ward, B. Feist, and B. J. Burke. 2019. Using hierarchical models to estimate stock-specific and seasonal variation in ocean distribution, survivorship, and aggregate abundance of fall run Chinook salmon. Canadian Journal of Fisheries and Aquatic Sciences.

Sigler, M. F., D. J. Tollit, J. J. Vollenweider, J. F. Thedinga, D. J. Csepp, J. N. Womble, M. A. Wong, M. J. Rehberg, and A. W. Trites. 2009. Steller sea lion foraging response to seasonal changes in prey availability. Marine Ecology Progress Series 388:243–261.

Sinclair, E. H., D. S. Johnson, T. K. Zeppelin, and T. S. Gelatt. 2013. Decadal Variation in the Diet of Western Stock Steller Sea Lions (*Eumetopias jubatus*). NOAA Technical Memorandum NMFD-AFSC-248.

Sinclair, E. H., and T. K. Zeppelin. 2002. Seasonal and Spatial Differences in Diet in the Western Stock of Steller Sea Lions. Journal of Mammalogy 83(4): 973-990

Smout, S., C. Asseburg, J. Matthiopoulos, C. Fernández, S. Redpath, S. Thirgood, and J. Harwood. 2010. The functional response of a generalist predator. PLoS ONE 5.

Sobocinski, K. L., C. M. Greene, J. H. Anderson, N. W. Kendall, M. W. Schmidt, M. S. Zimmerman, I. M. Kemp, S. Kim, and C. P. Ruff. 2021. A hypothesis-driven statistical approach for identifying ecosystem indicators of coho and Chinook salmon marine survival. Ecological Indicators 124:107403.

Steingass, S. 2017. Dietary Composition of Four Stocks of Pacific Harbor Seal (*Phoca vitulina richardii*) In the Northern California Current Large Marine Ecosystem As Synthesized From Historical Data, 1931–2013. Northwestern Naturalist 98:8–23.

Swan, J.G. 1870. The Indians of Cape Flattery, at the entrance to the Strait of Fuca, Washington Territory. Smithsonian Contributions to Knowledge 16.

Sweeney, J. M., and J. T. Harvey. 2011. Diet estimation in California sea lions, *Zalophus californianus*. Marine Mammal Science 27:279–301.

Tarroux, A., J. Bêty, G. Gauthier, and D. Berteaux. 2012. The marine side of a terrestrial carnivore: Intra-population variation in use of allochthonous resources by Arctic foxes. PLoS ONE 7(8). e42427. doi:10.1371/journal.pone.0042427

Terraube, J., and B. Arroyo. 2011. Factors influencing diet variation in a generalist predator across its range distribution. Biodiversity and Conservation 20:2111–2131.

Thomas, A. C., B. E. Deagle, J. P. Eveson, C. H. Harsch, and A. W. Trites. 2016. Quantitative DNA metabarcoding: Improved estimates of species proportional biomass using correction factors derived from control material. Molecular Ecology Resources 16:714–726.

Thomas, A. C., B. Deagle, C. Nordstrom, S. Majewski, B. W. Nelson, A. Acevedo-Gutiérrez, S. Jeffries, J. Moore, A. Louden, H. Allegue, S. Pearson, M. Schmidt, and A. W. Trites. 2022. Data on the diets of Salish Sea harbour seals from DNA metabarcoding. Scientific Data 9:1–12.

Thomas, A. C., S. N. Jarman, K. H. Haman, A. W. Trites, and B. E. Deagle. 2014. Improving accuracy of DNA diet estimates using food tissue control materials and an evaluation of proxies for digestion bias. Molecular Ecology. 23(15):3706–3718

Thomas, A. C., M. M. Lance, S. J. Jeffries, B. G. Miner, and A. Acevedo-Gutiérrez. 2011. Harbor seal foraging response to a seasonal resource pulse, spawning Pacific herring. Marine Ecology Progress Series 441:225–239.

Thomas, A. C., B. W. Nelson, M. M. Lance, B. E. Deagle, and A. W. Trites. 2017. Harbour seals target juvenile salmon of conservation concern. Canadian Journal of Fisheries and Aquatic Sciences 74:907–921.

Thompson, S. A., W. J. Sydeman, J. A. Thayer, A. Weinstein, K. L. Krieger, and D. Hay. 2017. Trends in the pacific herring (*Clupea pallasii*) metapopulation in the California current ecosystem. California Cooperative Oceanic Fisheries Investigations Reports 58:77–94.

Tirasin, E. M., and T. Jørgensen. 1999. An evaluation of the precision of diet description. Marine Ecology Progress Series 182:243–252.

Tollit, D., L. Fritz, R. Joy, K. Miller, A. Schulze, J. Thomason, W. Walker, T. Zeppelin, and T. Gelatt. 2017. Diet of endangered steller sea lions (*Eumetopias jubatus*) in the Aleutian islands: New insights from DNA detections and bioenergetic reconstructions. Canadian Journal of Zoology 95:853–868.

Tollit, D. J., G. J. Pierce, K. A. Hobson, W. D. Bowen, and S. J. Iverson. 2010. Chapter 9: Diet. Pages 191–221 *in* I. L. Boyd, W. D. Bowen, and S. J. Iverson, editors. Marine Mammal Ecology and Conservation : A Handbook of Techniques. Oxford University Press, Incorporated.

Tollit, D. J., A. D. Schulze, A. W. Trites, P. F. Olesiuk, S. J. Crockford, T. S. Gelatt, R. R. Ream, and K. M. Miller. 2009. Development and application of DNA techniques for validating and improving pinniped diet estimates. Ecological Applications 19(4):889-905.

Tollit, D. J., M. A. Wong, and A. W. Trites. 2015. Diet composition of Steller sea lions (*Eumetopias jubatus*) in Frederick Sound, southeast Alaska: A comparison of quantification methods using scats to describe temporal and spatial variabilities. Canadian Journal of Zoology 93:361–376.

Treaty of Neah Bay. Jan. 31, 1855. 12 Stat., 939. Accessed: https://goia.wa.gov/tribal-government/treaty-neah-bay-1855

Trites, A. W., and D. G. Calkins. 2008. Diets of mature male and female steller sea lions (*Eumetopias jubatus*) differ and cannot be used as proxies for each other. Aquatic Mammals 34:25–34.

Trites, A. W., and R. Joy. 2005. Dietary analysis from Fecal Samples: How many scats are enough? Journal of Mammalogy 86:704–712.

Trites, A. W., and D. A. S. Rosen. 2019. Synthesis of Scientific Knowledge and Uncertainty about Population Dynamics and Diet Preferences of Harbour Seals, Steller Sea Lions and California Sea Lions, and their Impacts on Salmon in the Salish Sea. Technical Workshop Proceedings. May 29-30, 2019. Marine Mammal Research Unit, University of British Columbia, Vancouver, B.C.

Trudel, M., J. P. Fisher, J. A. Orsi, J. F. T. Morris, M. E. Thiess, R. M. Sweeting, S. Hinton, E. A. Fergusson, and D. W. Welch. 2009. Distribution and Migration of Juvenile Chinook Salmon Derived from Coded Wire Tag Recoveries along the Continental Shelf of Western North America. Transactions of the American Fisheries Society 138:1369–1391.

Voelker, M. R., D. Schwarz, A. C. Thomas, B. W. Nelson, and A. Acevedo-Gutiérrez. 2020. Large-scale molecular barcoding of prey DNA reveals predictors of intrapopulation feeding diversity in a marine predator. Ecology and Evolution 10:9867–9885.

Waite, J. N., V. N. Burkanov, and R. D. Andrews. 2012. Prey competition between sympatric Steller sea lions (*Eumetopias jubatus*) and northern fur seals (*Callorhinus ursinus*) on Lovushki Island, Russia. Canadian Journal of Zoology 90:110–127.

Waite, J. N., L. P. Waits, M. Bozza, and R. D. Andrews. 2011. Differentiating between Steller sea lion (*Eumetopias jubatus*) and northern fur seal (*Callorhinus ursinus*) scats through analysis of faecal DNA. Molecular Ecology Resources 11:166–170.

Walters, C. J., M. K. McAllister, and V. Christensen. 2020. Has Steller Sea Lion Predation Impacted Survival of Fraser River Sockeye Salmon? Fisheries 45:597–604.

Weitkamp, L. A. 2010. Marine Distributions of Chinook Salmon from the West Coast of North America Determined by Coded Wire Tag Recoveries. Transactions of the American Fisheries Society 139:147–170.

Wessen, G. C., and D. R. Huelsbeck. 2015. Environmental and Cultural Contexts of Paleoshoreline Sites on the Northwestern Olympic Peninsula of Washington State. BC Studies:263–290.

Winship, A. J., A. M. J. Hunter, D. A. S. Rosen, and A. W. Trites. 2006. Food consumption by sea lions: Existing data and techniques:177–191.

Winship, A. J., A. W. Trites, and D. G. Calkins. 2001. Growth in body size of the steller sea lion (*Eumetopias Jubatus*). Journal of Mammalogy 82:500–519.

Womble, J. N., M. F. Sigler, and M. F. Willson. 2009. Linking seasonal distribution patterns with prey availability in a central-place forager, the Steller sea lion. Journal of Biogeography 36:439–451.

Womble, J. N., M. F. Willson, M. F. Sigler, B. P. Kelly, and G. R. van Blaricom. 2005. Distribution of Steller sea lions Eumetopias jubatus in relation to spring-spawning fish in SE Alaska. Marine Ecology Progress Series 294:271–282.

Wray, J. and the Olympic Peninsula Intertribal Cultural Advisory Committee, 2003. Native peoples of the Olympic Peninsula: who we are. University of Oklahoma Press.

Zaccarelli, N., D. I. Bolnick, and G. Mancinelli. 2013. RInSp: An R package for the analysis of individual specialization in resource use. Methods in Ecology and Evolution 4:1018–1023.

APPENDIX TABLE

Appendix Table A1: Otolith lengths and counts recovered from Steller sea lion scats. Standard length calculated from standard length regressions in (Harvey et al. 2000).

	Number of otoliths	Otolith length (mm)	Standard length (cm)
Walleye pollock (*Gadus chalcogrammus*)	106	5.46-16.63	9.88-34.90
Salmonid, unknown species *(Oncorhynchus* spp.)	21	3.53-4.11	
Pacific herring (*Clupea pallasii*)	18	1.6-4.06	6.53-19.42
Starry flounder (*Platichthys stellatus)*	5	3.16-7.34	3.25 - 17.25
American shad (*Alosa sapidissima*)	1	3.01	23.41
Shiner perch (*Cymatogaster aggregata*)	2	2.05-2.4	3.05-3.66
Coho salmon (*Oncorhynchus kisutch*)	2	3.29-3.93	12.92 -23.36
Lingcod (*Ophiodon elongatus*)	2	7.51-8.08	53.60 - 58.29
American shad (*Alosa sapidissima*)	1	3.01	23.41
Smelt, unknown (Osmeridae sp.)	1	1.43	
Eelpout (Zoarcid sp.)	1	3.53	