Harbor Seals in Hood Canal: Predators and Prey

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Abstract

Harbor Seals in Hood Canal: Predators and Prey

Joshua Michael London

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The foraging ecology and population dynamics of harbor seals (*Phoca vitulina richardsi*) were studied in Hood Canal, Washington from 1998 to 2005. Initial work was conducted in response to concerns over the potential impact seals may have on recovering populations of summer chum salmon. Direct observation of harbor seals consuming salmon within the inter-tidal regions of four rivers in Hood Canal were conducted from 1998-2001 and 2003. Seals were observed feeding on chinook, coho, pink, summer chum and fall chum salmon. Estimates of summer chum consumption by seals at each of the observation sites averaged 8.0% of returning adults across all sites and all years. The maximum percentage of returning chum consumed was 27.7% and the lowest was 0.84%. The number of seals observed foraging in the river for salmon averaged from two to seven seals. Summer chum populations in the study streams have increased over the time of the study to near historical highs. Because of this increase, the levels of predation observed are not believed to significantly impact the recovery of summer chum in Hood Canal. A protocol for extraction of DNA and identification of seal sex from scats was developed to examine differential diets between male and female harbor seals. Scats from both sexes contained similar levels of Pacific hake, but male scats contained more salmon and female scats contained more Pacific herring. In 2003 and 2005, mammal-eating killer whales foraged exclusively within Hood Canal for 59 and 172 days respectively. Bio-energetic models and boat based observations were used to estimate harbor seal consumption by killer whales and, in both years, the predicted consumption was approximately 950 seals. However, aerial surveys conducted following the two foraging events have not detected a significant decline in the harbor seal population.

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Introduction

The foraging ecology of pinnipeds has been the subject of scientific investigations for many years because pinnipeds are often perceived as being in competition with human fisheries. Pinniped-fishery interactions are often classified as either operational or biological (Baraff and Loughlin 2000). Operational interactions refer to activities that result in a direct impact. Entanglement in fishing gear and other forms of incidental and directed take would be considered 'operational' interactions. Pinniped behavior can also result in a direct impact on a fishery. Seals and sea lions are known to remove fish from set-nets, commercial long-lines and recreational fishermen. Grey seals (*Halichoerus grypus*) in Britain have been implicated in a number of detrimental fishery interactions ranging from predation on free swimming and net-pen salmon to more indirect effects of driving fish away from nets and increasing the presence of cod worm in the Atlantic cod (Furness 2002; Harwood and Greenwood 1985). As pinniped populations have increased along the West coast of the United States, recreational fishermen commonly report loss of catch due to seals and sea lions removing fish from their lines (NMFS 1999). Charter boat companies in Washington, Oregon and California are often forced to change locales during a trip because of the presence of seals and sea lions (NMFS 1999).

Biological interactions can be categorized as either exploitative competition or interactive competition (Baraff and Loughlin 2000). Exploitative competition occurs when pinnipeds are in direct competition with fisheries for the same prey. Seal and sea lion populations in the northeast Pacific rely on ground-fish, herring, salmon and squid as major components of their diet (Lowry and Frost 1985). These same species are also large components of commercial fishery operations and pinnipeds are often perceived as being in direct competition with these fisheries. Interactive competition is less direct and examples include pinnipeds abandoning a foraging area due to disturbance, reduced foraging efficiency or disruption of foraging patterns (Baraff and Loughlin 2000).

However, pinniped-fishery interactions must also be examined with some perspective. During the late nineteenth and early twentieth centuries, many pinniped stocks throughout the world were hunted to a small fraction of their former population levels

(Beddington et al. 1985) for both the fur trade and to reduce fishery interactions. From early in the twentieth century until the passage of the federal Marine Mammal Protection Act of 1972 (MMPA), all West coast states, including Washington, had an active pinniped control unit and bounty program for the removal of pinniped species. Significant conservation efforts in the last century and passage of laws such as the MMPA have allowed many pinniped populations to rebound; however many remain critically low or are declining (VanBlaricom et al. 2001).

In recent years, the west coast of the United States has seen the emergence of a new category of pinniped-fishery interaction. Since the passage of the MMPA, populations of California sea lions (*Zalophus californianus*) and Pacific harbor seals (*Phoca vitulina richardsi*) have experienced dramatic increases throughout the West Coast, and may be at their highest levels in several centuries (NMFS 1999). The increase in pinniped populations has coincided with dramatic decreases of many marine and anadromous fish populations (NMFS 1997b). A number of these populations have declined to a point where they have been listed, or are under consideration for listing, as endangered or threatened under the federal Endangered Species Act of 1973 as amended (ESA). There has been a growing concern throughout the West Coast that pinnipeds, while not likely the cause of any decline in salmonids, have the potential to affect the recovery of many threatened and endangered salmonid stocks (NMFS 1999).

Ballard Locks

The importance of understanding the potential impact pinnipeds could have on declining salmonid populations came to the forefront when California sea lions were consistently observed foraging on returning winter steelhead at the Ballard Locks in Seattle, Washington. The situation in Ballard was ongoing from the mid-1980s through 1995 and was well documented (NMFS 1995; 1999). At the peak of predation activity in this location, close to sixty percent of the returning winter steelhead run was being consumed by sea lions. As the number of returning steelhead reached critically low levels, various non-lethal mitigating actions were employed in an attempt to reduce the level of predation on steelhead. By 1995, the non-lethal actions were determined to be

ineffective and, after a number of hearings and detailed investigations, the National Marine Fisheries Service (NMFS) issued a permit to the Washington Department of Fish and Wildlife (WDFW) for the lethal removal of individual pinnipeds under Section 120 of the MMPA.

Before lethal removal was implemented, however, arrangements were made to capture and transport the predatory sea lions to Sea World in Orlando, Florida for display and captive breeding. Since the individual sea lions were removed from the area, little or no predation has been observed. However, while there was an initial increase in numbers of returning steelhead in years directly following removal of predatory sea lions, the annual number of returning spawners remains critically low (WDFW unpublished data).

The situation at Ballard is not unique. Predation by California sea lions on salmonids has also been observed at the Willamette Falls area near Portland, OR (NMFS 1997a), below and in the fish-way at Bonneville Dam on the Columbia River and below Merwin Dam on the Lewis River (pers. comm. Steve Jeffries, Washington Department of Fish and Wildlife, Tacoma, WA). Additionally, harbor seals have been observed feeding on out-migrating salmon smolts in the Punteldge River on Vancouver Island in British Columbia (Yurk and Trites 2000) and adult salmon in coastal rivers of Oregon, Washington and California (NMFS 1997b).

Of the numerous lessons and knowledge gained from the situation at Ballard, three stand out. First, managers and researchers need to take a proactive approach by initiating studies to understand the impact of pinnipeds on a declining population before actually reaching the level of threatened or endangered. Section 120 of the MMPA requires that a “significant negative impact” to the threatened or endangered population be demonstrated in a quantitative manner. This requires several years of research, and if not done prior to the population reaching a critically low level, valuable time may be spent investigating the impact instead of implementing management action that could lead to recovery (NMFS 1997b).

The second lesson is, while there are certainly unique aspects to the Lake Washington Ship Canal, and other locations that allow unprecedented levels of pinniped predation on returning salmonids, it is reasonable to expect analogous situations to exist in a more natural environment. Pinniped haul-outs throughout the west coast often occur at or near the mouths of rivers that support a number of declining salmonid populations (NMFS 1997b). At any such estuarine or nearshore area where fish passage is constrained by natural or artificial barriers, there are possibilities for predation to occur (NMFS 1999). The key question, however, is not whether predation is occurring, but rather if predation is negatively affecting the ability of a particular salmonid population to recover from low numbers. Additionally, any negative affect pinniped predation may be having on the recovery of a particular salmonid stock should be considered within the context of a myriad of other factors such as habitat degradation, harvest, climate change, pollution and ocean conditions. Predator prey relationships between salmon and pinnipeds, such as harbor seals, have been evolving for thousands of years and the complexity of that relationship must be considered when looking to situations like Ballard as examples. California sea lions, the salmonid populations, and any artificial barriers and human modifications there now did not exist in the same location until relatively recent.

Another theme that emerged was the methods used to evaluate the food habits of pinnipeds were inadequate to effectively estimate and predict the impact such predation may or may not have on a particular salmonid population (NMFS 1997b). Scat collection and analysis would have to be combined with other techniques and technologies such as direct surface observation for predation events, genetic analysis, and comprehensive population modeling approaches.

Pinniped Food Habits

Research on the food habits of California sea lions and Pacific harbor seals in the past has shown that they are opportunistic consumers, with the majority of their diets consisting of seasonally and locally abundant prey. For sea lions and harbor seals in the greater Puget Sound and Straight of Georgia, this translates into a diet, while diverse, made up mostly of Pacific hake (*Merluccius productus*) and Pacific herring (*Clupea*

harengus pallasi) (Calambokidis et al. 1989; NMFS 1995; 1997b; Olesiuk et al. 1990). In some locations, significant pinniped predation has been reported on returning adult salmonids (NMFS 1995; 1997a; b) or out-migrating salmon smolts (Yurk and Trites 2000). While salmonids have been found in the diet of local pinnipeds, the vast majority of studies were not designed to address impacts of pinniped predation on specific salmonid populations. Previous studies were mostly conducted on an opportunistic basis, not necessarily within a period of high salmonid abundance, and focused on the collection of scat and analysis of prey remains. Understanding the role of salmonids in the foraging ecology of seals and sea lions is especially problematic because, until recently, only otoliths (fish ear bones) were used to identify prey items. Otolith bones from salmonids are more fragile than other bones and are not often recovered in pinniped scats. In fact, recent analysis of the frequency of occurrence (FO) of salmonids in the diet of harbor seals in Hood Canal has shown an approximate five-fold increase in the percentage of scats containing salmonids when all structures are used compared to only otoliths (Lance unpublished). Other factors, involving gut retention, travel time between haul-outs and foraging locations, and the potential that scats collected are not a representative sample of a population, may further limit the ability to interpret the role of salmonids in the diet of pinnipeds with scat analysis alone.

NMFS Investigation and the West Coast Pinniped Study

In February of 1997, NMFS completed a review of scientific information on impacts of California sea lions and Pacific harbor seals on West Coast salmonids (NMFS 1997b). This report discussed themes previously mentioned as a result of the Ballard situation and identified a number of locations where there was a potential for pinnipeds to impact recovery of declining salmonids. This led to an initial allocation of resources to the Oregon Department of Fish and Wildlife (ODFW) to begin evaluating the use of direct surface observations as a way of estimating predation rates of pinnipeds on salmonids. Studies were expanded to include researchers from WDFW, California Department of Fish and Game (CDFG) and NMFS in 1998. All participating researchers coordinated efforts through the Pacific States Marine Fishery Commission and adapted a similar approach and methodology for each specific site and question of interest.

Research Presented

Research presented in this dissertation reflects much of the work conducted collaboratively with the Washington Department of Fish and Wildlife between 1998 and 2005. Results are presented in three chapters:

(1) Harbor Seals and Salmon in Hood Canal: Estimates of Predation by Harbor Seals on Threatened Summer Chum Salmon,

(2) The Impact of Two Extended Transient Killer Whale Foraging Events on the Harbor Seal Population in Hood Canal.

(3) The Use of Genetic Scat Analysis in Pinnipeds for Determination of Sex and Species Specific Food Habits,

Hood Canal - Harbor Seals and Summer Chum

Hood Canal is a fjord-like body of water that lies just east of the Olympic Peninsula and makes up the western most portion of Puget Sound in Washington State. Five major rivers (Quilcene, Dosewallips, Duckabush, Hamma Hamma and Skokomish rivers) originate from headwaters in the Olympic Mountains and flow into Hood Canal. Each river supports runs of various salmonid species including chinook (*Oncorhynchus tshawytscha*), coho (*O. kisutch*), chum (*O. keta*) and pink (*O. gorbuscha*). Steelhead (*O. mykiss*) and sea run cutthroat (*O. clarkii*) are present as well. In recent years, many salmonid runs have declined sharply, with several runs (chinook, summer chum and Dosewallips pinks) listed in the 1992 Salmon and Steelhead Stock Inventory (WDF et al. 1993) as critical or depressed.

Summer chum salmon in Hood Canal were listed as ‘Threatened’ under the ESA in 1999 (WDFW et al. 2000). Escapement and abundance estimates were precipitously low in the late 1980s and 1990s and were at a fraction of historic levels (WDFW et al. 2000). Summer chum salmon return to Hood Canal streams in August and September and are genetically distinct from Fall chum runs that return in late October and November (WDFW et al. 2003). The reasons for the decline in abundance are not fully understood,

but are likely related to by-catch, habitat loss and reduced ocean productivity (WDFW et al. 2000).

Of the six extant native summer chum stocks within Hood Canal, four (Quilcene, Dosewallips, Duckabush and Hamma Hamma) return to rivers that have harbor seal haul-outs associated with the lower tidal areas. Harbor seal populations in Hood Canal (approx. 1000 animals) and the rest of Washington state are considered abundant and healthy (Jeffries et al. 2003). Each of the five main haul-out sites in Hood Canal can range from approximately 50-250 seals during August and September (Calambokidis et al. 1990). This temporal and spatial overlap has lead to concern over the impact seal predation might have on the conservation and recovery efforts of summer chum in Hood Canal. Previous studies in Hood Canal have examined harbor seal diet by identification of otoliths found in scat (Calambokidis et al. 1978; Calambokidis and McLaughlin 1987). Pacific hake composed more than eighty percent of harbor seal diet based on scat collected at the Skokomish, Duckabush, and Dosewallips rivers and Quilcene Bay. In these studies, salmon was not found to be a significant portion in the diet of harbor seals in Hood Canal, however; the use of only otoliths for identification of prey is known to under-represent prey species with more fragile otoliths like salmonids.

Most studies of pinniped foraging behavior are limited because their foraging activity occurs at depth and is unobservable by researchers. The typical solution is to rely on archival tags to provide information on diving behavior and inferences to feeding activities. The small, relatively shallow tidal streams in Hood Canal that were the focus of our research efforts allowed an unprecedented view of this unique seal foraging behavior. Information presented in this chapter will focus on seal behavior and quantitative estimates of seal predation on summer chum based on surface observations conducted in 1998, 1999, 2000, 2001 and 2003.

Killer Whales in Hood Canal

Prior to 2003, killer whales were considered a rare occurrence in Hood Canal. Acoustic recordings from the US Navy submarine base at Bangor and reports from long time residents of Hood Canal, suggest both resident and transient type killer whales have been

present in Hood Canal. However, the frequency of reports is extremely low. Additionally, when whales have been observed in Hood Canal it has been for no more than one or two days.

In January of 2003, 11 transient-type, mammal-eating killer whales arrived in Hood Canal and remained exclusively within the canal for 59 days. The extended stay of such a large group of transients was considered atypical. Anecdotal observations suggest these whales were feeding on harbor seals and bio-energetic estimates suggested more than half of the seal population should have been removed. Subsequent aerial surveys in 2003 and 2004 have not shown a significant decline in seal abundance, and, in January of 2005, six different transient type killer whales arrived in Hood Canal and stayed for 172 days. Bio-energetic and observation estimates suggest a similar level of removal occurred during the 2005 event, but harbor seal surveys in 2005 also do not exhibit a sharp decline. This chapter will review details of these two extended foraging events, parameters and predictions of the bio-energetic model and an evaluation of harbor seal aerial surveys to determine the population impact of these killer whale predation events.

Scat Genetics

In recent years, analysis of prey remains found in scats has become the method of choice for investigation of pinniped diets. In most locations, scats can be collected in large numbers with relative ease and minimal disturbance. Scat analysis, however, does have limitations. Biases associated with recovery and identification of otoliths and bones from some prey species prevent reliable use of scats for more than generalized characterization of diet (Cottrell et al. 1996; Harvey 1989; Lance et al. 2001). Investigation of more detailed aspects of pinniped foraging, such as sexual variation in diet, would require more intrusive actions such as enemas, lavaging, or examination of stomachs from harvested individuals. Additionally, collections of scats from haul-outs shared by more than one pinniped species are often confounded because scats cannot be separated visually or based on collection location. Genetic analysis of scat material is an alternative non-invasive technique that would allow individual scats to be classified

based on sex, species or individual identification of the source animal (Kohn and Wayne 1997).

Genetic scat analysis has been employed in a number of terrestrial mammalian studies (Ernest et al. 2000; Farrell et al. 2000; Kohn et al. 1999; Morin et al. 2001; Wasser et al. 1997), yet its application to pinniped scat analysis has been limited (Reed et al. 1997). This chapter will focus on development of an efficient and reliable protocol for extraction of pinniped DNA from scats. While extracted DNA can provide the basis for a variety of genetic investigations (Kohn and Wayne 1997), here the focus will be on amplification of sex specific and species specific markers and their potential use for examination of variation in diet between sexes and species. This methodology, when combined with standard protocols for identification of prey remains from scat (Lance et al. 2001), can provide researchers with new insights into the foraging ecology of pinnipeds.

The chapters presented here are written as independent manuscripts that will be submitted to peer-reviewed journals for publication. Therefore, there may be overlap and repetition of themes covered in this introduction and other chapters.

Chapter 1. Harbor Seals and Salmon in Hood Canal: Estimates of Predation by Harbor Seals on Threatened Summer Chum Salmon

Introduction

Since passage of the Marine Mammal Protection Act (MMPA) in 1972, many populations of pinnipeds along the West Coast of the United States have rebounded to historic highs. Harbor seals in Washington (Jeffries et al. 2003), Oregon (Brown et al. 2005) and California (Carretta et al. 2005) are all at or near estimated carrying capacity. California sea lions throughout the West Coast are growing exponentially (Carretta et al. 2005) and expanding their range. The increasing numbers are likely a testament to the adaptability and productivity of these pinniped species and protection afforded them under the MMPA. From early in the twentieth century until the passage of the MMPA, Washington and other states maintained active pinniped control and bounty programs for the removal of pinniped species. Pinniped control programs were largely based on the view that seals and sea lions were direct competitors to commercial and recreational fisheries and removal of predators would benefit fisheries. Now that pinniped populations have rebounded, they are, once again, a focus of concern among fisheries managers. This time, however, the concern is over the impact increasing seal and sea lion populations may be having on a major conservation effort to recover declining populations of salmon.

Coincident to increases in pinniped numbers over the last 30 years has been a significant decline in the number of returning salmon in Washington, Oregon and California. Several of the declining populations have been listed as ‘Threatened’ or ‘Endangered’ under the Endangered Species Act (ESA). The extent to which pinnipeds are a hindrance to recovery of these declining salmon populations is not known. There have been a few case studies where pinniped predation on salmon has accounted for losses of a large fraction of returning adults (Ballard, Willamette) (NMFS 1995; 1997a) or out-migrating smolts (Puntledge River, British Columbia) (Yurk and Trites 2000). However, the majority of pinniped diet studies in locales where salmon are present have shown salmon to be a minor component of the year-round diet of seals and sea lions (NMFS 1997b).

Pinnipeds are likely not the reason for the widespread decline in salmon populations, but could be responsible for inhibiting recovery in localized small populations.

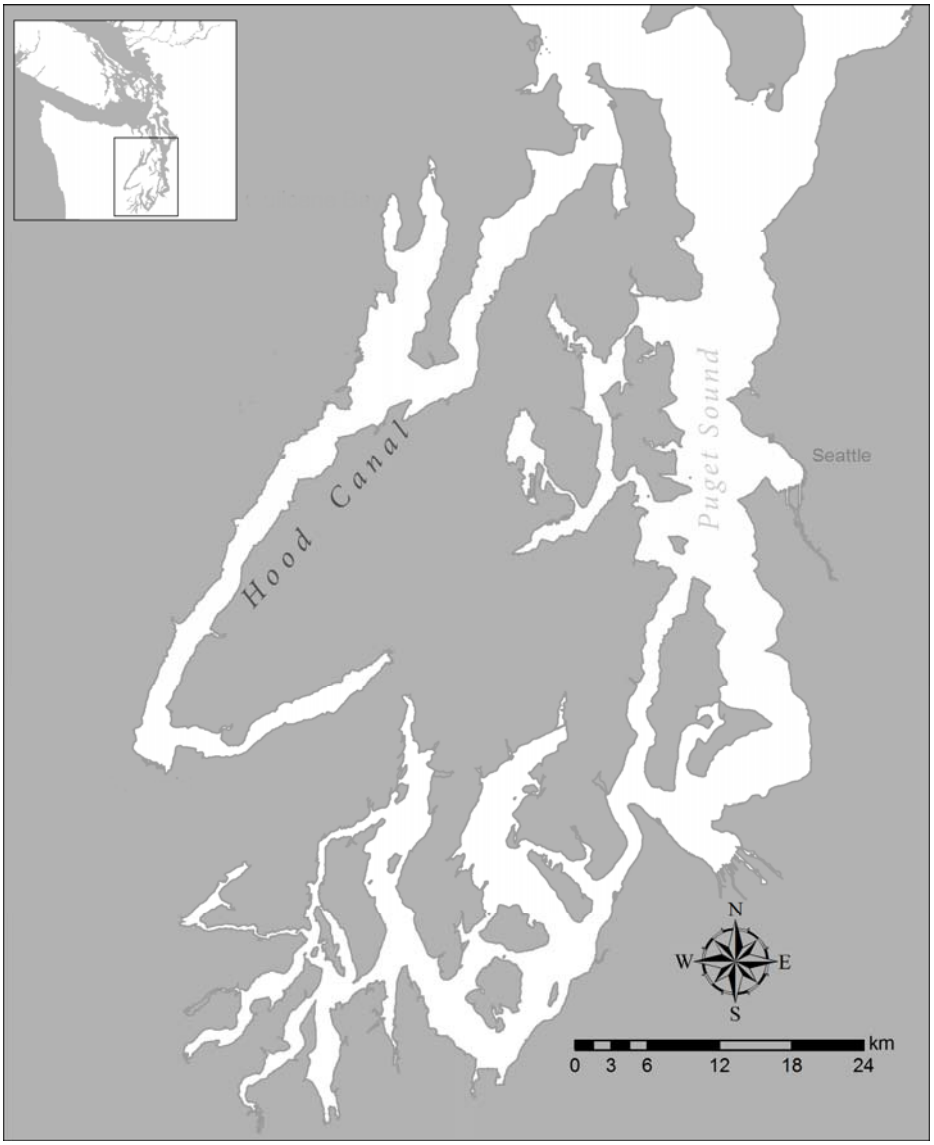

Figure 1.1 Map of the Puget Sound and Hood Canal region in Washington State, USA.

Summer chum salmon in Hood Canal (Figure 1.1) were listed as 'Threatened' under the ESA in 2001. Escapement and abundance estimates were precipitously low in the late 1980s and 1990s (WDFW et al. 2003) and were at a fraction of historic levels (Figure

1.2). Summer chum salmon return to Hood Canal streams in August and September and are genetically distinct from Fall chum runs that return in late October and November (WDFW et al. 2003). The reasons for the decline in abundance are not fully understood, but are likely related to by-catch, habitat loss and reduced ocean productivity (WDFW et al. 2000).

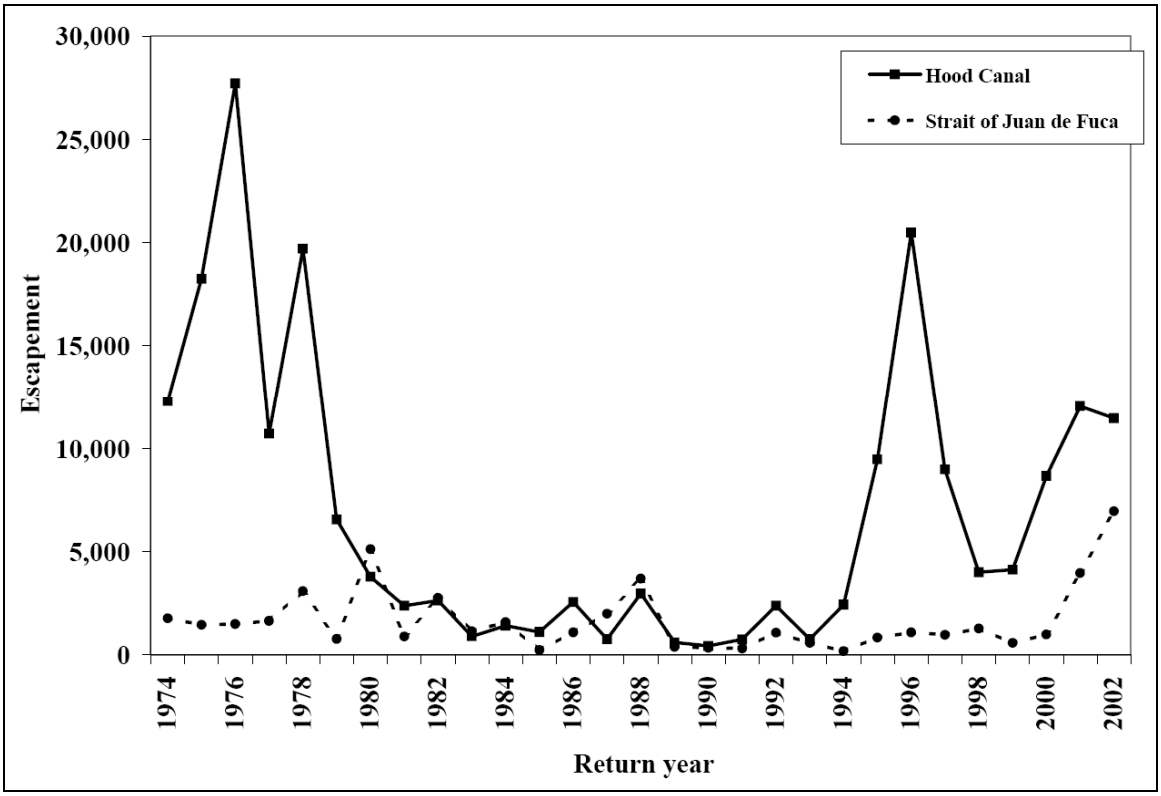

Figure 1.2 Summer chum salmon escapements to Hood Canal and Strait of Juan de Fuca streams, 1974 through 2002 (WDFW 2003).

Of six extant native summer chum stocks within Hood Canal, four (Quilcene, Dosewallips, Duckabush and Hamma Hamma) return to rivers that have harbor seal haul-outs associated with their lower tidal areas. The total population of seals in Hood Canal is approximately 1,000 (Jeffries et al. 2003) and counts at each of these four haul-out sites can range from approximately 50-250 seals during August and September (Calambokidis et al. 1991). This temporal and spatial overlap has lead to concern over

impacts seal predation might have on conservation and recovery efforts of summer chum in Hood Canal.

In most cases, the use of direct observation of harbor seal foraging behavior is limited because the majority of foraging events take place several meters underwater. However, in those situations where seals are taking advantage of high prey concentrations in a limited area, direct observation can provide significant insight into consumption rates and foraging behavior. Harbor seal predation on smolt and adult salmonids is one such scenario and observation of surface predation events is the basis for estimates of adult salmonid consumption at four river systems in Hood Canal, Washington between 1998 and 2003.

Harbor seal surface predation events are denoted as those times when a seal brings a captured salmon to the surface for consumption. This is due, in large part, to the physical size of the salmonid prey. Many returning salmon are close to one-third the body length of an average harbor seal and significantly larger than other prey items found in the diet of Hood Canal seals. Captured salmon are often brought to the surface for killing and subsequent consumption, however; consumption underwater is possible and likely more common for smaller sized species (e.g. pink salmon) that require less handling time.

Each of the four major river systems on the west side of Hood Canal (Figure 1.3) offers unique access to the mouth and estuarine areas where salmonid surface predation events can be observed. These areas are all less than one square kilometer in size and, for the most part, can be effectively covered visually by one or two observers. Additionally, low flow levels and relatively shallow waters that typically exist during the observation season provide observers with the ability to track seals underwater by following their characteristic surface wake. Major harbor seal haul-outs are located at the mouth or within estuarine areas of each river. This, combined with concentrations of returning adult salmon in the estuary, resulted in a significant proportion of salmonid predations occurring within an observable area.

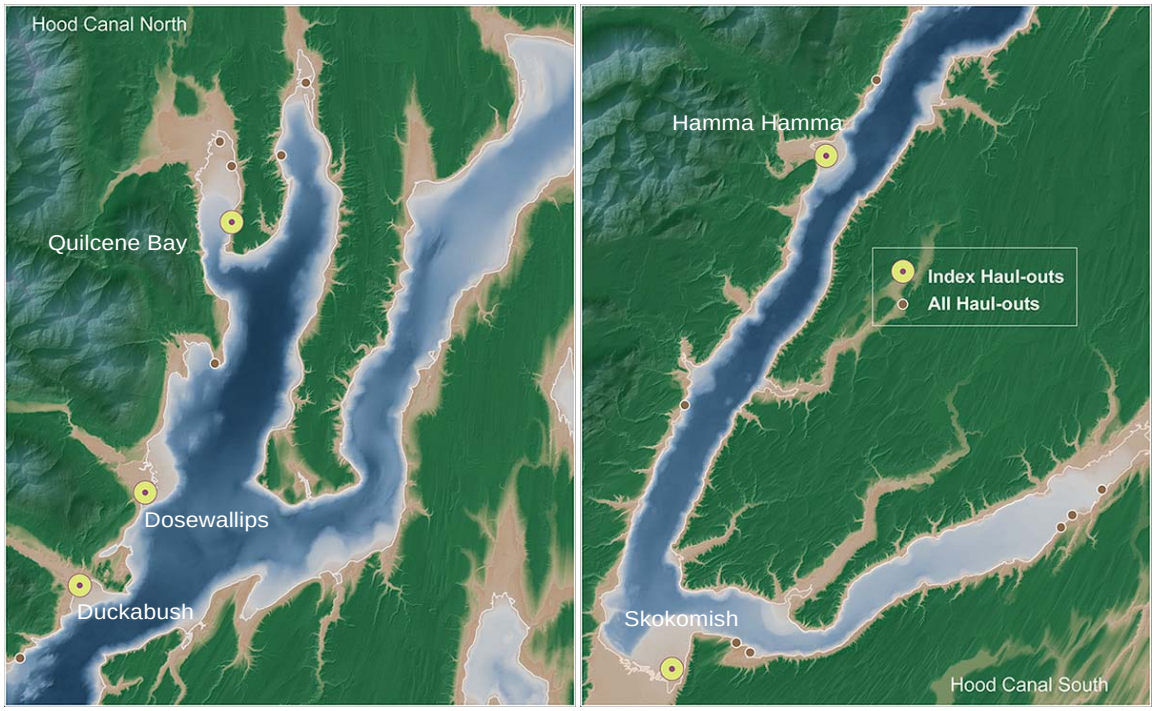

Figure 1.3 Map of North Hood Canal (left) and South Hood Canal (right) showing the locations of the observation sites and harbor seal haul-outs in Hood Canal.

Methods

Surface Observations

Field observations were conducted in Hood Canal to record surface predation events on returning adult salmon from vantage points off the mouths of the Quilcene (Big and Little), Dosewallips, Duckabush, and Hamma Hamma rivers. Observations were also conducted at the Skokomish River in 1998 and 1999, but data from the Skokomish is not presented here. Duration of field observation activity has varied from year to year. In 1998, observations were conducted from the first week of September through the second week in November. 1999 observations started the third week in August and concluded after the first week of November. 2000 observations started the same week as in 1999, but were finished the last week of October. 2001 and 2003 followed similar time spans as the 2000 season; however, observations were limited to the Dosewallips and Duckabush rivers. Differences in observation schedules were due in large part to an

increased understanding of salmonid abundance and timing, weather and tidal conditions at observation sites, and a desire to increase efficient use of research time and money.

In 1998 and 1999, a non-stratified cluster sampling regime was employed, consisting of 3 six-hour periods randomly sampled across 3 days each week (Sunday-Saturday). Approximately 300 hours of observation were conducted at each of the river mouths in 1998 and 1999. Additionally, a second observation site was added to the Duckabush location in 1999 to address predation events occurring upstream from the Highway 101 Bridge that were not viewable from the lower river site. For each sampling week, selection of specific sites for conducting surface predation observations was made randomly and scheduled in advance. Each daily observation period was scheduled to begin either 30 minutes after sunrise or end 45 minutes before sunset to allow adequate ambient light for observations. Observations were made from either a 16 ft tower blind (Dosewallips, Duckabush, and Hamma Hamma), or ground vantage point (Quilcene Bay) which allowed viewing of predation events within and around the lower main channel and tidal areas of each river.

In 1998 and 1999, daily observation periods lasted a total of 6 hours from arrival. The first 20 minutes following arrival were spent organizing equipment, data forms and setting up for observations. This was followed by three 100-minute observation periods with a 20 minute break between each. Weather, overall visibility conditions, maximum number of seals foraging in the river, and total number of salmonid predations was recorded for each 100-minute observation.

The observation sampling-scheme was significantly altered for the 2000, 2001 and 2003 seasons. Preliminary data analysis from 1998 and 1999 indicated that predation rates were not constant across the entire tidal cycle with a majority occurring on an incoming tide. Stratified random sampling allowed concentration of observation effort on those times during which predations were more likely. The 2.5-month observation season was divided into five two-week periods. Two-week sampling periods were divided into two strata. The first stratum consisted of four-hour periods during which a maximum percentage of predations had been observed at a particular site during the previous two

field seasons. The second stratum consisted of the remaining time and was divided into 2-hour blocks. The first stratum consisted of those periods occurring during observable daylight hours. As in 1998 and 1999, observable daylight hours were denoted as 30 minutes after sunrise to 45 minutes before sunset.

Once the two strata were determined, four maximum predation periods were chosen at random for each two-week period. Each of these periods was observed in its entirety. For the second stratum, two days were chosen at random and two two-hour periods were randomly chosen for each day. These two-hour periods did not overlap and occurred outside the maximum predation stratum.

The focus of the observer, during all years, was to cover the area encompassing each site where predation by seals was possible. Binoculars and spotting scopes were used to scan the area for pinniped presence and detection of predation events. Locations of predation events were identified and recorded based on a gridded location map of each observation site. Each observer documented any predation or foraging event on the data form. Observers noted time, location, number of seals involved, species of salmon (if possible), a confidence factor of 1-5 for prey identification, and a variety of possible behaviors (e.g. chase, competition, partial consumption).

Nighttime Predation

Nighttime predation observations were conducted during the 2000, 2001 and 2003 field seasons. Observations were only done at the Duckabush River and were paired with scheduled daytime observations in order to provide statistically comparable information on the potential differences in seal activity between daytime and nighttime. Nighttime observation periods were selected to occur during four-hour high predation strata that existed between sunset and sunrise, and within 24 hours of a similar daytime observation. This allowed a paired analysis for comparison of mean number of predations and number of foragers. Observers were positioned at the Mouth Site and at the Highway 101 Bridge over the Duckabush River and observations were made using an ITT 5001P head-mounted night vision goggle with a slip-on 3X magnifier lens. All

attempts were made to identify and record predation activity following the same protocols used during daylight hours.

Night-vision equipment provided adequate clarity for observers to identify seal movements and behavior within those areas of the river that were illuminated by light from the moon or ambient sources. Areas covered by shadows (eg. bank edge areas below the bridge, areas in the immediate vicinity of the bridge, and most area upstream from the bridge) were too dark for detailed observations of predation events and other seal behavior.

Calculation of Predation Estimates

Estimates of salmonid predation in 1998 and 1999 were determined through use of a cluster sampling estimator. Each week served as an individual stratum with the primary units being the random sample of three of seven days per week and the sub-units of three 100-minute observation periods on those days.

The additional up river site at the Duckabush River (1999-2001, 2003) was treated as independent during calculation of predation estimates. Observers were in constant radio communication to prevent any overlap in recorded predation events. After estimates were analyzed, they were combined with the lower site (mouth) for comparison and analysis with fish abundance numbers. Data collected in 2000, 2001 and 2003 relied on a stratified random sampling estimator to calculate the number of predations for each bi-week period. Weekly estimates from 1998 and 1999 were combined into bi-weekly estimates for comparison across all years.

Allocation of Salmon Predations to Species

Observation of potentially significant predation of summer chum by harbor seals has raised the importance of allocating salmonid predations to individual salmonid species. Allocation of salmonid predations is especially problematic in Hood Canal because those species with reduced populations (summer chum, chinook) overlap in timing with more abundant species (coho, pink, fall chum). The extent to which seals are selective towards one species over another is not known and likely not something that will be determined

without exceptional effort. Given these constraints, two scenarios with two different analysis assumptions were explored for estimating predation impact on individual salmon species. Each of these analysis scenarios is focused on determining the impact on summer chum and is thus reflective of only the time during which summer chum were present in each system (approximately August to mid-October).

Scenario I assumes there is no selection by harbor seals for or against summer chum in relation to other salmonids, and the percentage of summer chum predations is equal to the percentage of summer chum present. This scenario is the most objective and parsimonious, however, estimates of availability derived from in-river spawner counts likely do not fully represent the dynamic nature of species availability in the lower reaches and tidal estuaries of these rivers.

Scenario II assumes predations identified by observers to species are reflective of all salmonid predations and this percentage is used to estimate the impact of predations on summer chum. This scenario relies heavily on the ability of observers to identify predations to salmonid species, that their identifications are unbiased, and that each species is equally identifiable. Most predations occur at a fair distance from the observer, last only a few seconds, are mostly underwater and often provide little information that would allow an observer to determine species. Additionally, the differences in size, color and life history of each salmonid species (e.g. chum vs. coho) are variable and an assumption that each species is equally identifiable is debatable.

Scenario II relies on the ability of each observer to identify predations to salmon species consistently and without bias. All adult salmon undergo color and morphology changes prior to spawning. Some species, such as chum and pink, begin the transformation to their spawning phase at or just before their entry into freshwater. Coho and chinook species tend to retain their non-spawning ‘bright’ coloration and morphology well after their entry into freshwater. Salmon species in their ‘bright’ saltwater morphology and coloration are virtually indistinguishable at any significant distance. Positive identification of species while in this ‘bright’ phase usually requires careful examination of gum coloration, spot patterns or scale size. Once salmon undergo transformation to

their spawning morphology, identification can be possible at distance because of conspicuous coloration patterns or morphological changes. Male pink salmon acquire a large hump on their back; chum salmon develop green and purple coloration patterns; and coho salmon take on a deep red coloration. Because observation areas in this study are tidally influenced, both phases are possible for each species along with various transitional phases. This leads to a situation whereby a predation event involving a 'bright' salmon can only be identified as an 'unidentified salmon', a salmon in a transitional phase may be identifiable to species, and a salmon in the spawning phase is most easily identified to species. The different life histories and ecology of salmon species present in Hood Canal result in unequal probabilities of identification in the study observation areas. The unequal likelihood of positive species identification is further complicated by differences in handling time for each species. Because of these limitations with Scenario II and the more parsimonious nature of Scenario I, all calculations of the percentage of summer chum consumed by seals will be based on the relative percentage of summer chum available.

Estimates of Summer Chum Abundance

Detailed estimates of harbor seal predation on returning salmon do not provide any quantifiable information on population level impacts without similarly detailed estimates of summer chum escapement. WDFW generously provided bi-weekly estimates of summer chum abundance for each river with predation observation sites. Estimates were calculated based on spawning curves generated from spawner surveys done in each river. Since spawner surveys were conducted upstream from the area covered during predation observations, a correction for travel time to the spawning ground was included to provide estimates that reflected abundance of each species in the lower river observation areas. Details of the techniques and assumptions used to calculate escapement estimates are presented in a WDFW technical report (Adicks et al. 2004).

Results

Seal Behavior

The Dosewallips and Duckabush rivers provided observers the best opportunity to record seal behavior. Maximum numbers of seals foraging for salmon at each site for 1999-2001 and 2003 were recorded for every observation period. The average value for the maximum number of foragers at each site was calculated for each year (Table 1.1). The mean value for the Dosewallips across all years was 2.75 (se=0.41) seals and 4.77 (se=0.68) seals in the lower reaches of the Duckabush river. The maximum number of seals observed at the Dosewallips was eight and as many as fifteen were observed at the Duckabush. The number of individual seals actively foraging for salmon in the lower reaches of these rivers at any one time represents less than five percent of the total population of seals that use nearby haul-outs.

Table 1.1 Average value for the maximum number of harbor seals actively foraging for salmon during an observation. 2000, 2001 and 2003 values are calculated from the high-predation strata only. Standard deviations based on the Poisson distribution are presented in parentheses.

Site	1999	2000	2001	2003
Dosewallips River	2.11 (1.45)	2.61 (1.62)	2.35 (1.53)	3.95 (1.99)
Duckabush River	2.81 (1.68)	4.85 (2.20)	5.67 (2.38)	6.45 (2.54)

The presence of seals foraging in the lower reaches is closely related to tidal stage. The six-hour observations conducted in 1998 and 1999 were scheduled without respect to tidal stage. Start times for all predations at each site were examined with respect to time from the nearest high tide. At each site, the majority of salmon predations by harbor seals occurred on an incoming tide and within a few hours of high tide. This relationship between harbor seal behavior and tide cycles is a result of two key elements. First, the small, low-flow nature of these streams restricts movement of seals into the lower reaches where salmon are more vulnerable to predation. Seals are unable to move upstream until tides reach one or two meters above sea level. Second, the movement of salmon into these streams occurs mostly on the incoming tide.

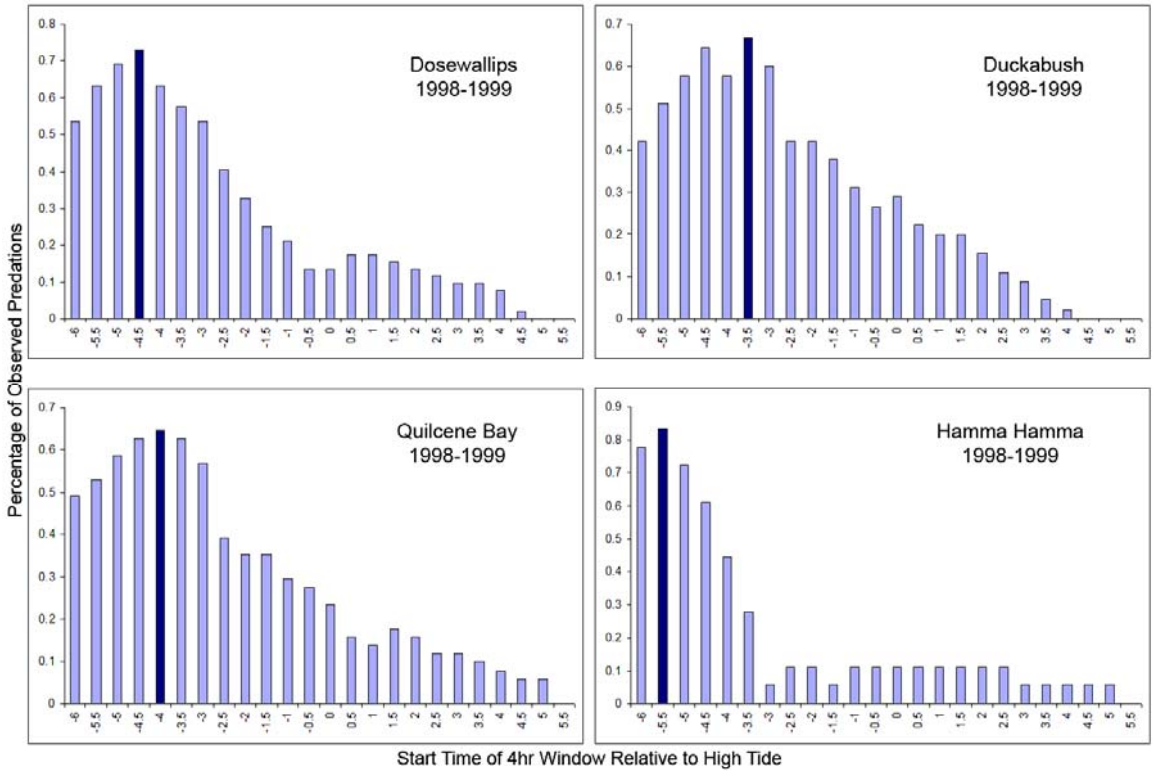

Figure 1.4 Percentage of observed salmon predations occurring within overlapping four-hour bins relative to high tide at four locations in Hood Canal. The 'zero bin' represents the percentage of predations occurring from high tide to four hours after high tide.

The tidal cycle was divided into 30-minute segments that ranged from six hours before to six hours after high tide. Recorded predations were binned into overlapping 4-hour windows to determine the four hour window with the highest proportion of observed predations (Figure 1.4). The window with the highest proportion was used as the defining element for the stratified sampling design employed in 2000, 2001 and 2003.

Predation Estimates

Harbor seal predation on salmonids was observed at all five sites. On a few occasions, California sea lion predation on salmonids was observed off the Hamma Hamma River. Estimates of salmonid predations presented here only include predation attributed to harbor seals. Harbor seals were observed preying on chum and coho salmon in Quilcene

Bay; chum, coho, and pink at the Duckabush River; chum, coho and pink at the Dosewallips River; chum, pink and chinook at the Hamma Hamma.

Most predations at the Dosewallips and Duckabush rivers were within 50-75m of the observation platform and provided observers with the best observation conditions for identifying captured salmon to species. For 2000, 2001 and 2003, the average percentage of predations identified to species was only 51.4% (n=106) for the Dosewallips and 52.3% (n=273) for the Duckabush. The low percentage of predations identified to species indicates positive species identification is not common and, more importantly, feedback from observers each field season suggests salmon species are not equally identifiable.

Unequal probability of positive identification is related to the presence of conspicuous characteristics for each salmon species and total handling time of the seals. Average handling times for each species from the Dosewallips and Duckabush rivers across the 2000, 2001 and 2003 field seasons were calculated based on the recorded start and end time for predation events. Seals averaged 8.89 minutes (s.d. = 10.23, n = 83) for chum and 8.15 minutes (s.d. = 7.05, n = 37) for coho, while pink salmon predations lasted only 4.90 minutes (s.d. = 3.14, n = 92). Predations recorded as ‘unidentified salmon’ had an average handling time of 5.16 minutes (s.d. = 5.87, n = 191). The shorter handling time for pink predations would result in less opportunity for an observer to note distinguishing characteristics for species identification. Pink salmon (present only in 2001 and 2003) may be under-represented in the subset of predation events with positive species identification.

Table 1.2 Comparisons between paired day-time and night-time observations at the Duckabush River over three years (2000, 2001, 2003) of observations. The presented p-values are from a two-tailed pairwise t-test.

	Salmon Predations		Max Number of Foragers	
	Day-time	Night-time	Day-time	Night-time
Mean per Observation	2.75	0.95	4.08	4.1
Standard Deviation	2.93	1.44	2.73	1.61
Paired Observations (n)	60	60	60	60
p-value	< 0.001		0.96	

The ability to discern detail during nighttime observations was significantly affected by cloud cover, moon stage and angle of the moon. The ability of researchers to confirm predation events was, therefore, significantly compromised compared to daytime estimates. Differences in both the number of predations observed and maximum number of foragers between day and night were evaluated with a paired t-test for sample means. Results indicate a significant reduction in the number of predations observed at night compared to the day (Table 1.2). Given the inherent differences in observability between day and night, even with advanced night vision goggles, comparison of predation rates is not informative. However, there was no significant difference in the number of foragers present in the river.

Examination of telemetry data from animals tagged with VHF and sonic tags showed the same animals were present in the river during both day and night. Additionally, a logarithmic relationship (R^2 = 0.426, p=0) exists between the number of observed salmon observations and the maximum number of foragers observed during an observation (Figure 1.5). Comparing maximum number of foragers may provide a better index for assessing predation activity between day and nighttime periods.

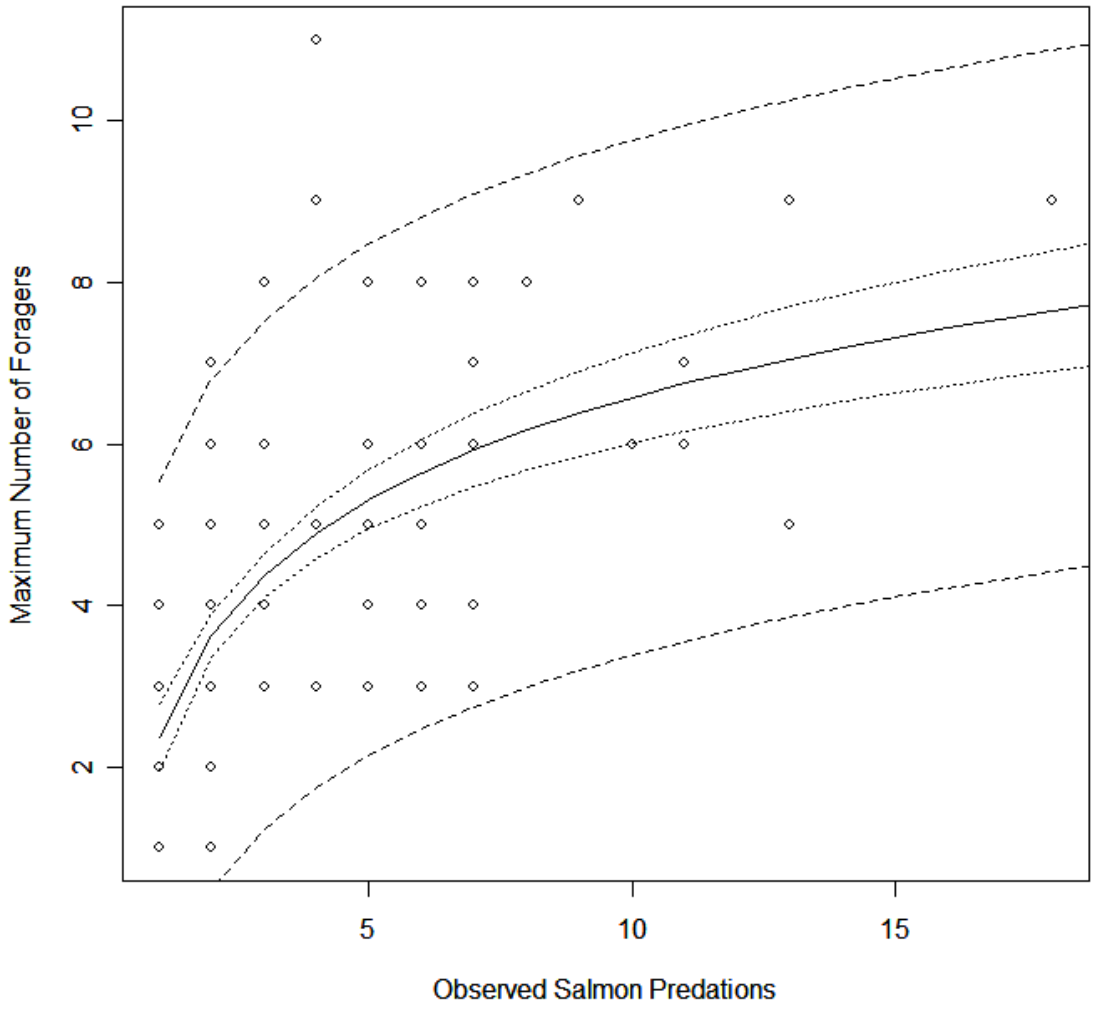

Figure 1.5 Scatter plot of the Maximum Number of Foragers vs. Observed Salmon Predations for day-time observations during high predation strata in 2000, 2001 and 2003. The solid line indicates the logarithmic regression model. The narrow and wide bands indicate the confidence interval and prediction band.

Estimates of harbor seal predation impacts on Hood Canal summer chum runs were calculated at each site for each year using Scenario I for allocation of species (Table 1.3). Harbor seal consumption calculations are done with an assumption that predation rates observed during daylight hours are consistent across the entire 24 hours. The largest estimate of absolute consumption occurred at the Duckabush River in 2003 with

166 summer chum consumed. The lowest absolute consumption was in 1998 at the Hamma Hamma River with only five summer chum consumed. The greatest and lowest estimated percentage of returning summer chum consumed occurred at the Dosewallips. In 1998, 27.7% of summer chum returning to the Dosewallips River were consumed by harbor seals. Yet, in 2001, only 0.84% of the run was consumed. The average, across all sites and years, was 8.0% (se=2.06) of the returning summer chum consumed.

Table 1.3 Estimates of predation by harbor seals on summer chum at sites in Hood Canal, assuming seals consume salmon species in proportion to their relative abundance and that night predation rates are equal to those observed during daylight hours.

Site	Est. S. Chum Predations	S. Chum Escapement	% of Run	CV
Year: 1998				
Quilcene	66	1922	3.44	0.213
Dosewallips	91	329	27.7	0.178
Duckabush	26	226	11.3	0.264
Hamma Hamma	5	106	4.38	0.535
Year: 1999				
Quilcene	43	2976	1.43	0.396
Dosewallips	19	351	5.46	0.271
Duckabush	10	93	10.4	0.209
Hamma Hamma	6	234	2.45	0.272
Year : 2000				
Quilcene	92	5469	1.68	0.082
Dosewallips	87	1293	6.76	0.066
Duckabush	132	494	26.8	0.082
Hamma Hamma	21	202	10.2	0.067
Year: 2001				
Dosewallips	8	990	0.84	0.064
Duckabush	40	944	4.22	0.031
Year: 2003				
Dosewallips	159	7065	2.25	0.032
Duckabush	166	1873	8.84	0.034

The wide variation in seal consumption between sites and across years (Figure 1.6) is due in large part to the differences in year to year salmonid escapement. In 1999, only 93 summer chum returned to spawn at the Duckabush River. That same year, nearly 3,000 summer chum returned to the Big Quilcene River. Furthermore, by 2003, the escapement

estimate for the Duckabush River had grown by twenty-fold. Variation in escapement of other salmon species that overlap temporally with summer chum also plays an important factor in each river. Under the Scenario I assumption of consumption in proportion to abundance, species that return in high numbers during the summer chum run (i.e. pink salmon) act as a buffer to summer chum consumption. Pink salmon only return to Hood Canal streams during odd years (1999, 2001 and 2003 in this study). The estimates of seal predation on summer chum tend to show higher percentage consumption in even years when pink salmon were not present.

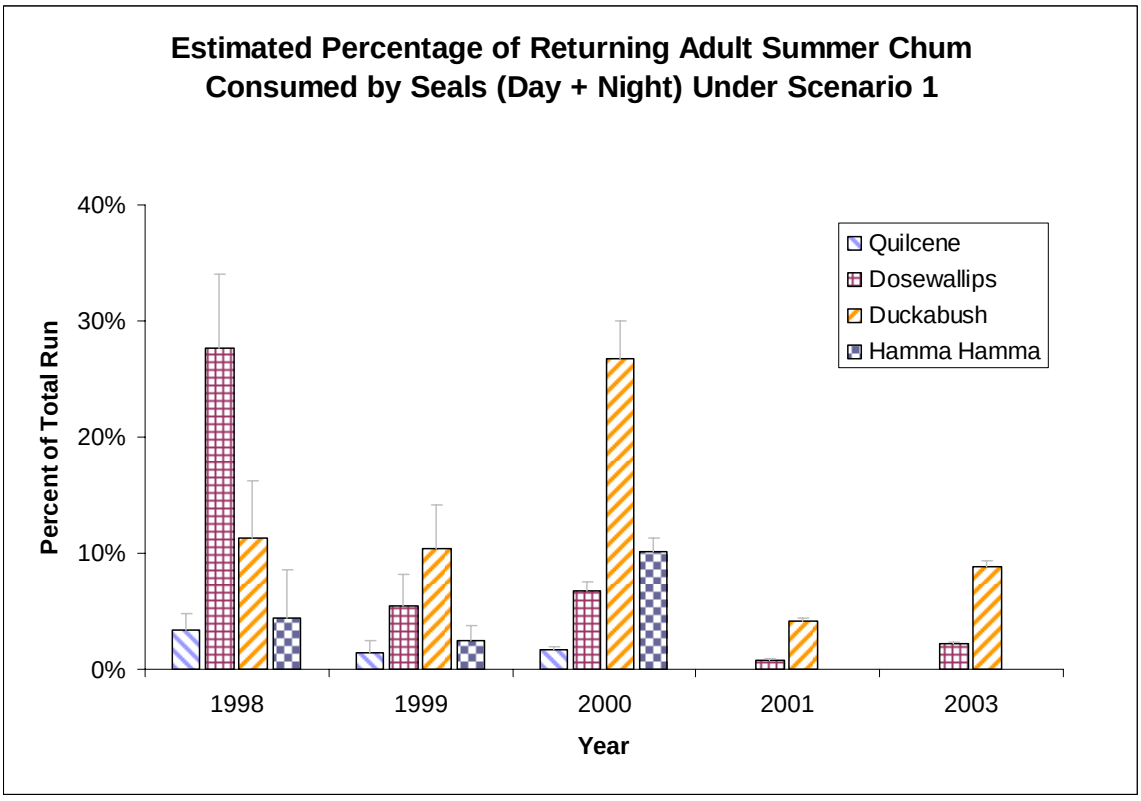

Figure 1.6 Estimated percentage of adult summer chum consumed by harbor seals at four sites in Hood Canal across four years under scenario 1 and assuming night predation rates are equal to those observed during daylight hours. Error bars indicate the upper 95% confidence interval.

Variance Calculation and Confidence Intervals

Variance calculations and 95% confidence intervals were calculated for the total predation estimate during the summer chum run at each site in each year. It should be noted that this does not include any variance that might be associated with salmonid abundance estimates. Ranges presented here only represent variance associated with the estimated total predation based on surface observations. Coefficients of variation were calculated for each site in each year (Figure 1.7). The benefits of the stratified random sampling approach used in 2000, 2001 and 2003 can be seen in reduced CV values for those years compared to 1998 and 1999.

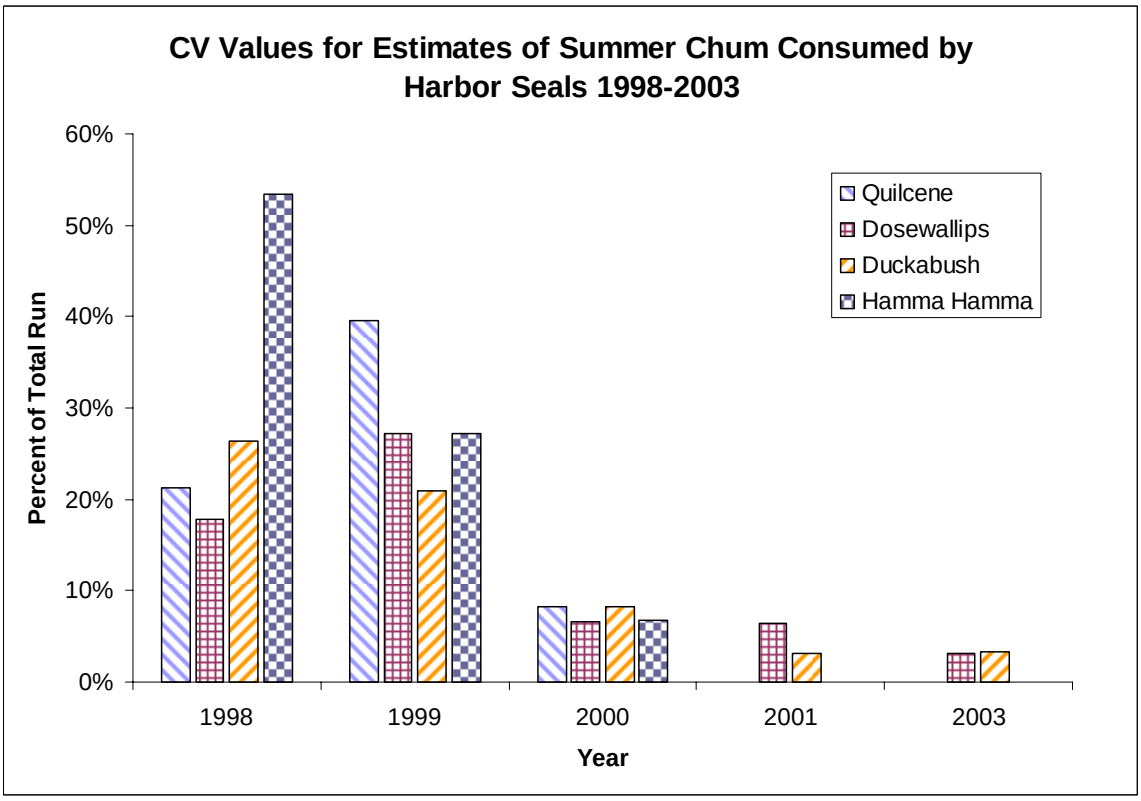

Figure 1.7 Coefficient of variation values for the estimates of summer chum consumed by harbor seals in Hood Canal. 1998 and 1999 observation scheduling was done irrespective of tidal stage. 2000, 2001 and 2003 observations were scheduled under a stratified sampling protocol and observation effort was skewed towards periods of higher predation rates.

Discussion

Assessing Population Impacts of Seal Predation

Seal predation on summer chum and other salmonids was observed at a higher rate than initially expected, but predation estimates alone cannot provide enough insight to evaluate impacts on recovery of Hood Canal summer chum populations. The estimates of predation rates from this study should be evaluated in context with the productivity potential of summer chum. Summer chum were not historically studied for critical population parameters such as age composition and recruits per spawner. Lack of data for these critical parameters has made it difficult to assess impacts of harbor seal predation and other forms of mortality on the population dynamics of summer chum.

Under the summer chum conservation plan (WDFW et al. 2003), recovery criteria include the achievement of an average of 1.6 recruits per spawner over the eight most recent brood years, with values of 1.2 or less occurring in no more than two of the eight years. Under the plan, fishery-related mortality is expected to be no more than 16.7%. Using the highest reported predation estimate from this study (27.7% at the Duckabush in 2000) and the maximum expected incidental-catch mortality, a population with a spawner-recruit ratio of 1.6 would still increase by 15.6%. In reality, the likelihood of these maxima occurring in the same year is minimal. Results from this study indicate that in most years at most sites, harbor seal predation amounts to less than 8% of the returning adults. Natural productivity would need to consistently fall below 1.1 recruits per spawner before harbor seal predation could impact recovery. However, as summer chum production and abundance fall, the functional response of harbor seals is likely to result in reduced predation rates. Harbor seals are opportunistic, generalist predators and likely have a type three functional response to the presence of salmonid prey (Middlemas et al. 2006). Their main diet in Hood Canal is composed of more consistently abundant and predictable prey such as hake and herring. With readily available alternative prey, the few seals involved in salmon predation are not likely to remain focused on salmon at low densities.

Examination of the annual escapement trend for summer chum in Hood Canal over the time of the predation study does suggest the observed level of predation has minimal impact on recovery of summer chum. Returns of summer chum to Hood Canal streams increased each year from 1998-2003 and, in 2004, was the highest on record (Adicks et al. 2004; WDFW et al. 2003). Extinction risk for summer chum in the four river systems in this study is now listed as moderate to low (WDFW et al. 2003). The age of returning chum salmon can be anywhere between two and five years with the majority returning as age three or four (WDFW et al. 2000). Therefore, the increased returns in 2001-2004 represent the production from adults subjected to predation from 1998 to 2000. The highest levels of predation observed during the study were 28% at the Dosewallips in 1998 and 27% at the Duckabush in 2000.

A natural broodstock supplementation program has been in employed in recent years for some of the critically low populations of summer chum in Hood Canal (WDFW et al. 2003). However, only one of the study streams (Hamma Hamma) has been included in the supplementation program. It is assumed increasing returns in the study streams in recent years are a result of natural summer chum production. Additionally, it is important to realize that results presented here account for only predation occurring within the lower reaches of spawning rivers. While we feel this predation accounts for the majority of harbor seal consumption in Hood Canal, it does not reflect any predation that occurs in the open water region of Hood Canal.

It is also important to consider potential indirect impacts seal behavior may have on production of summer chum in Hood Canal. Summer chum spawn in the lower reaches of these rivers and some of the spawning area is accessible by harbor seals at higher tide levels. Seal presence and harassment of spawning summer chum may have indirect implications on reproductive behavior and success. Such indirect impacts were not addressed by this study and no quantifiable data exists for proper examination. However, we can say the frequency is high enough that future studies and population viability exercises should include some low level of indirect effect.

The rebound of pinniped populations along the West Coast of the United States should be considered a large conservation success. High profile situations like the Ballard Locks in Seattle and Bonneville Dam on the Columbia River and, regular interactions between pinnipeds and commercial and recreational fishermen, have led to a miss-characterization of seals and sea lions as problem predators. This study demonstrates that, at least for Hood Canal, harbor seal predation, while important for managers to account for, is not an important priority in long term conservation efforts. The levels of summer chum predation estimated are not consistently high enough to result in a meaningful impact on summer chum production in Hood Canal.

The majority of seals in Hood Canal are relying on hake and herring as their stable diet. Over two-thousand scats were collected across all the sites in Hood Canal from 1998-2003 and 74% contained remains of Pacific hake and 43% Pacific herring; salmon was

present in 27% of the scats (Lance unpublished). When FO is normalized to add up to 100%, salmon only accounts for 14% of the harbor seal diet. Harbor seals are opportunistic predators and when a large biomass of prey such as salmon is available some individuals will choose to take advantage of the resource. In Hood Canal, an even smaller subset of the population has adapted to focus almost exclusively on salmon within the lower reaches of these small rivers.

The conclusion that harbor seal predation has minimal impact on the recovery potential of summer chum in Hood Canal does not mean predation by harbor seals, and other pinnipeds, at terminal areas can be ignored by resource managers. This is especially important in areas where pinniped predation is known to be at consistently high levels from year to year. Recent increases in the presence of California sea lions feeding on salmon near dams along the Columbia River may be such a situation. It is essential, in these areas, that some level of pinniped predation be incorporated into management plans and harvest allocation scenarios. Without adequate set-asides for pinnipeds and other natural predators, managers may unwittingly prescribe harvest levels beyond sustainability. Unfortunately, accurate estimates of pinniped predation require long-term studies and results are not likely applicable across different regions and species. In those areas where pinniped predation is believed to be a critical issue, robust monitoring programs should be implemented to estimate the level of predation and results from other studies should be incorporated into a precautionary approach to resource allocation.

Seals and salmon in Hood Canal have developed a balanced trophic relationship over thousands of years. As long as higher priority efforts in salmon conservation to protect and restore habitat and reduce by-catch and over-fishing are successful, there should be enough salmon for harbor seals and other predators, including recreational, commercial and tribal fisheries. The increasing trend in escapement of summer chum to Hood Canal during the previous six years may be evidence of successful recovery planning efforts on the part of resource managers and local citizens to restore a declining population to historical levels.

Chapter 2. Impacts of Two Extended Foraging Events by Mammal-Eating Killer Whales on the Population of Harbor Seals in Hood Canal, Washington.

Introduction

The theory that transient-type, mammal-eating killer whales may be responsible for declines of pinniped populations in the Eastern North Pacific has garnered significant debate (Springer et al. 2003; Williams et al. 2004). This hypothesis stems from the conclusion that predation by a few killer whales is responsible for a dramatic decline in abundance of sea otters in the Aleutian Islands (Estes et al. 1998). Springer et al. (2003) expanded the top-down effect of killer whale predation to other pinniped species in the North Pacific. Their theories rely heavily on assumptions regarding daily metabolic requirements of wild killer whales and their functional response to prey populations. These assumptions and analyses were outlined in Williams et al. (2004). Unfortunately, opportunities to verify these assumptions with empirical data from wild populations are limited. Mammal-eating killer whales are characterized by discreet behavior and spend much of their time in remote locations not frequented by researchers. Therefore, knowledge of killer whale intake rates is limited to small datasets of mostly opportunistic data or extrapolations from captive killer whales or other large terrestrial carnivores.

One might imagine an ideal situation whereby daily requirements of killer whales could be estimated from a wild population. Under this scenario, a group of whales would be confined to a specific geographic area over a certain period of time. These whales would then be provided with a known amount of prey. As long as no additional prey were added to the area or removed by any means other than killer whale predation, differences in prey abundance would provide an estimate for daily energetic requirements and insights into the potential impact killer whales might have on isolated pinniped populations throughout the Eastern North Pacific. Additionally, observations of killer whales while in the area would provide an independent assessment of prey consumption.

Two recent extended foraging events by killer whales in Hood Canal, Washington are a close representation of this ideal situation and have provided an unprecedented opportunity to empirically measure the impact of mammal-eating killer whales on a pinniped population. Hood Canal is an isolated 100km fjord on the west side of Puget Sound and supported an estimated population of 1068 harbor seals in 2002. Between 2 January and 3 March, 2003, eleven mammal eating killer whales foraged exclusively within Hood Canal. A second group of six mammal-eating whales were in Hood Canal for 172 days in 2005.

Three separate killer whale ecotypes are present in the marine waters of the Pacific Northwest. Fish-eating, resident-type orcas are believed to feed exclusively on fish and predominantly on salmon. Fish-eating killer whales have a strong matrilineal social structure and have been extensively studied throughout Washington and British Columbia for the past 30 years. Mammal-eating, transient-type killer whales are known to feed exclusively on other marine mammals (seals, sea lions, small cetaceans and some large whales). Mammal-eating orcas are less frequently observed, although an extensive photo-identification catalog does exist and many individuals have been photographed at least once. Offshore killer whales are a third distinct ecotype and are most commonly found away from coastal waters. Little is known about this ecotype, though preliminary studies indicate they may be feeding on high trophic level fish species.

Killer whales have not had a significant presence in Hood Canal within the past thirty years, although both mammal-eating and fish-eating killer whales have been previously observed in Hood Canal. For both types, occurrences have been extremely rare and for less than one or two days. A few acoustic recordings of killer whales from U.S. Navy operations in Hood Canal have been confirmed and identified from their unique acoustic dialect as fish-eating orcas.

Harbor seals are reported to be one of the preferred prey items for mammal-eating killer whales, and harbor seals are the only consistently abundant resident marine mammal species known to occur in Hood Canal. Regular aerial and ground counts of harbor seals in Hood Canal have been conducted since the late 1970s and the population, as a part of

the larger population of seals within the semi-enclosed marine waters of Washington, is believed to have stabilized at near carrying capacity in the mid-1990s (Jeffries et al. 2003). Tagging and telemetry studies conducted in Hood Canal and other areas of Washington indicate no significant movement of seals between areas and, therefore, any comparison of pre and post harbor seal relative abundance is likely not significantly compromised by emigration or immigration.

The unique nature of these two killer whale incursions to Hood Canal has provided an opportunity for empirical investigation into predation behavior of mammal-eating killer whales and their impacts on localized pinniped populations. In order to maximize this potential, a multi-faceted investigation was employed. The approach can be divided into three key areas. First, behavioral observations, mostly from the 2005 event, have provided opportunities to directly estimate killer whale consumption and document foraging behaviors of mammal-eating killer whales. Second, a quantitative analysis of harbor seal aerial survey counts over time provides a mechanism to evaluate expected population responses given the presence of killer whales. Third, bio-energetic modeling allows a more theoretical examination of the trophic impact of killer whales. Each facet provides an independent evaluation of the impact of killer whale predation on the population of harbor seals in Hood Canal. By comparing these estimates we not only gain insight into the trophic ecology of killer whales, but also the benefits and limitations of each approach.

Methods

Behavioral Observations

All behavioral observations were conducted under the authority of Scientific Research Permit No. 782-1719, issued by the National Marine Fisheries Service under the authority of the Marine Mammal Protection Act and the Endangered Species Act. Opportunities to observe killer whales in Hood Canal during 2003 were limited to a few days. Most of the 2003 field effort focused on documenting group structure through photo identification and understanding the spatial use of Hood Canal. All whales were

photographed from the left and right side and individual identification was determined from identification catalogs.

Hood Canal is populated with a number of shore-side residences and, because of its narrow, fjord-like geography, provides ample opportunity for residents of Hood Canal to observe killer whales. Observations by a few residents and a dedicated volunteer provided the best information on movement and behavior of the whales in 2003.

In 2005, a coordinated effort between three research groups, in addition to observations of local residents and volunteers, provided a better dataset for examining killer whale foraging behavior and their spatial use patterns. As in 2003, all whales were photographed from their left and right side and identified through comparisons with photographic catalogs. Nineteen boat-based observations were conducted to document predations and movements of the killer whales within Hood Canal. Each observation was done opportunistically given weather and researcher availability. All observations were conducted from 19-21 foot outboard powered vessels and all available resources were used to locate the whales as soon as possible. Mobile phone coverage within Hood Canal allowed local residents to quickly communicate sightings to researchers and recent postings to internet distribution lists often provided critical information on sightings. When recent sightings were not available, a search transect of Hood Canal was conducted from the research boat until the whales were located.

Once whales were located, an initial GPS location was recorded and a trackline record was initiated. Whales were counted and visually identified to confirm all individuals were present. In general, the focus of the observation boat after first contact was to follow and record confirmed predation events without altering whale behavior. Under these circumstances, the general protocol was for the research boat to remain approximately 100m behind the whales. Fast acceleration and ‘leap frog’ actions were typically avoided and all attempts were made to minimize any effects the research boat might have on the behavior of the whales. For some of the observation periods, other objectives, such as collection of biopsy samples or prey remains, required temporary departures from this protocol.

A strict protocol was employed for identification and confirmation of predation events. Whales were closely observed for any changes in their behavior that might indicate potential interactions with harbor seals or other prey. All predations were confirmed by the presence of prey remains in the water column, an oil-slick on the surface of the water or an observation of prey remains within the mouth of a whale. Additional behavioral clues, such as observed interactions with live seals on the surface, and the presence of diving gulls provided further evidence of predation activity but were not used as sole confirmation of a predation event. The GPS location of all predation events was recorded, and each predation event was considered complete when the whales returned to their nominal travel behavior.

In order to extrapolate observed predations to an estimate of killer whale consumption, observations would ideally be of equal length and scheduled randomly across time. However, the opportunistic constraints of our effort negated the ability to plan observations in advance. Additionally, time to first location for any given planned observation trip was not predictable. Therefore, all attempts were made to approximate a random and unbiased sample of time. When possible, the length of the observation period was pre-determined on commencement. This was done to avoid any bias that might occur if whale behavior was used as a determining factor. For instance, it would not be advisable to consistently end observations after a predation event or to continue an observation until a predation event occurred. Both situations would bias the final estimates towards a higher consumption rate.

For each observation, a predation rate (kills/hour) was calculated from the number of confirmed predations and the length of the observation. An average predation rate was extrapolated across the duration of killer whale presence in Hood Canal under two scenarios. Scenario 1 assumes predations only occurred during daylight hours. All observations were conducted during daylight hours only. Information on the behavior of mammal-eating killer whales at night is limited and the only available study suggests indications of lower activity levels at night (Baird et al. *in review*). For the daylight-only scenario, the average predation rate was only extrapolated across hours between sunrise

and sunset. Scenario 2 was evaluated under the assumption that predation rates observed during the day are representative of killer whale behavior across day and night. Under this scenario, the average predation rate was extended across all hours of the day.

Generalized Linear Model

Aerial counts done between 1996 and 2004 were assembled and incorporated into a generalized linear model (GLM) to evaluate the impact of killer whale consumption in 2003 on the harbor seal population of Hood Canal. Further details on the aerial survey protocol can be found in Jeffries et al. (2003). When available, all counts were done from photographs taken during the aerial survey. When photographs were not taken, counts recorded by the aerial observer were used.

Harbor seal haul out patterns are known to be influenced by tidal height, tidal stage, time of day and day of year. Historical observations in Hood Canal suggest harbor seals are more likely to haul out at high tide stages in mid-afternoon and during the pupping (August-October) and molting (September-November) seasons. All aerial surveys in Hood Canal between 1996 and 2004 were flown between August and November and within +/- 2 hours of high tide. Because surveys are limited to times when seals are expected to haul out in the highest proportions, the inclusion of tidal factors (stage and height) were not included in the final GLM analysis.

Four hypotheses on how the Hood Canal seal population has responded to killer whale predation can be expressed as different GLMs. The first hypothesis suggests ‘no effects,’ and that aerial counts are correlated with only ‘day of year’ and ‘haul-out site.’ This hypothesis also suggests the population is stable over time period from 1996 to 2002. The second hypothesis predicts a ‘year effect’: the population of seals in Hood Canal is changing on an annual basis. The third hypothesis is the ‘treatment’ and represents a stable population between 1996 and 2000 that then changed in 2003 due to killer whale predation. The final hypothesis is similar to the ‘treatment’ effect but allows for growth in the population between 2003 and 2004. To evaluate whether there was a reduction in seal abundance in 2003, the four model variants were compared using AIC model selection.

At the time of writing, the aerial surveys of Hood Canal for 2005 have been completed but counts from photographs have not been finalized. Once final counts are available, a similar GLM analysis will be conducted.

Bio-Energetic Monte Carlo Simulation

A bio-energetic model of killer whale consumption was developed to estimate the predicted number of seals consumed by killer whales during the extended stays in Hood Canal. Parameters for metabolic requirements for killer whales were selected from published literature and information on the caloric value of seals was derived from seals captured in Hood Canal, caloric analysis of seals from Washington state and values from published literature.

Caloric content of harbor seals was determined from two whole body carcasses collected in the Grays Harbor and south Puget Sound regions of Washington. Both animals were considered in healthy body condition at the time of death and were provided by the Washington Department of Fish and Wildlife.

Carcasses were ground whole in the food preparation area at United Farms in Graham, Washington. Homogenate was passed through the grinder twice to insure complete homogenization. Four approximate four ounce aliquots were taken from each homogenate and stored at -20 C. The grinder was washed and cleaned between each of the carcasses to minimize any cross contamination.

Calorimetric content was determined with a Parr 1425 semi-micro bomb calorimeter (Parr Instrument Company, Moline, Illinois). Two 10g sub samples from each specimen were dried to constant mass at 50 C. Constant mass was reached when the percent change in mass was less than 0.2% in a 24-hour period. Sub-samples were further homogenized with mortar and pestle and an approximate 0.10g pellet was used in the bomb calorimeter. Caloric content was determined and converted to wet weight values based on sample moisture loss during drying.

The equation for determining total caloric requirements of the mammal-eating killer whales in Hood Canal:

(Whale kcal/kg/day) x [(Adult Male Mass x N_m whales) + (Adult Female - Subadult Mass x N_f whales) + (Juvenile Mass x N_j whales)] x (t Days)

Where N_m = number of Adult Males, N_f = number of Adult Females and Subadults, N_j = number of Juveniles, and t = the number of days present in Hood Canal

The caloric value of harbor seals in Hood Canal can be determined from

(Seal kcal/kg) x Seal Mass x Whale Assimilation Value

Whale requirements divided by the caloric value of harbor seals results in a predicted number of harbor seals consumed. This value, however, does not accurately reflect uncertainty around any of the parameters. Therefore, a Monte Carlo simulation was used to include uncertainty in the final estimate.

Table 2.1 Parameter values and distributions used in the Monte Carlo simulation of Killer Whale consumption of Harbor Seals in Hood Canal, Washington.

Parameter	Source	Range	Distribution
Whale Requirements	Williams et al. (2004)	55 kcal/kg/day	Fixed
Adult Male Mass		4200-7000 kg	Uniform
Adult Female-Subadult Mass		2100-3500 kg	Uniform
Juvenile Mass		1365-2275 kg	Uniform
Harbor Seal Caloric Content	Perez	2500-3800 kcal/kg	Uniform
Harbor Seal Mass	WDFW unpub. data	50kg	Normal with s.d. = 7
Assimilation Value	Williams et al. (2004)	0.85	Fixed
Number of Days		59	Fixed

A range of values was used for each parameter in the model (Table 2.1). A value of 55 kcal/kg/day is the median value reported (51-59) by Williams et al. (2004) for metabolic requirement of mammal eating killer whales. Ranges for killer whale mass were determined from reported values and consultation with other killer whale biologists. The harbor seal caloric content range includes values determined from the analysis of seals from Washington as well as reported values by Perez (1990) for ringed seals. The harbor seal mass value is a weighted average of non-pup, non-pregnant harbor seals captured in

Hood Canal between 1998 and 2002 (n=175). Non-pup, non-pregnant weights are used to best represent the available prey between January and June. The assimilation value of 0.85 is similar to values reported by Williams et al. (2004).

The bio-energetic model was calculated 15,000 times with new parameter values chosen from the listed ranges each time. A distribution of simulation outcomes and the median outcome along with 2.5 and 97.5 percentiles were calculated.

Results

Behavioral Observations

The killer whales present in 2003 and 2005 represent different individuals that are of no known relation. In 2003, the group consisted of 11 individuals (T14, T74, T73, T73a, T73b, T73c, T77, T77a, T77b, T123, and T123a) of which 2 were adult males (T14 and T74), 7 were sub-adults or females and 2 were juveniles (T73c and T77b). In 2005, six whales were present (T71, T71a, T71b, T124a, T124a1, T124a2) and the group was composed of two adult females (T71 and T124a) and their two offspring. With the exception of whale T14 (2003), these whales have limited to no sighting history in Washington state. The longest and most consistent sighting record of individuals from both groups comes from areas of northern Southeast Alaska (pers. comm. Jan Straley, University of Alaska Southeast, Sitka, AK).

Opportunities for detailed observations of the group in 2003 were limited to a few boat-based observations and sighting reports from residents of Hood Canal. All eleven whales were observed to use the entire expanse of Hood Canal and were most often observed as either one large group or two smaller groups of 5-6 whales. No confirmed predations were observed during boat-based research observations, however, several residents did report sightings of harbor seal predations and a few of those observations were confirmed with photographic documentation.

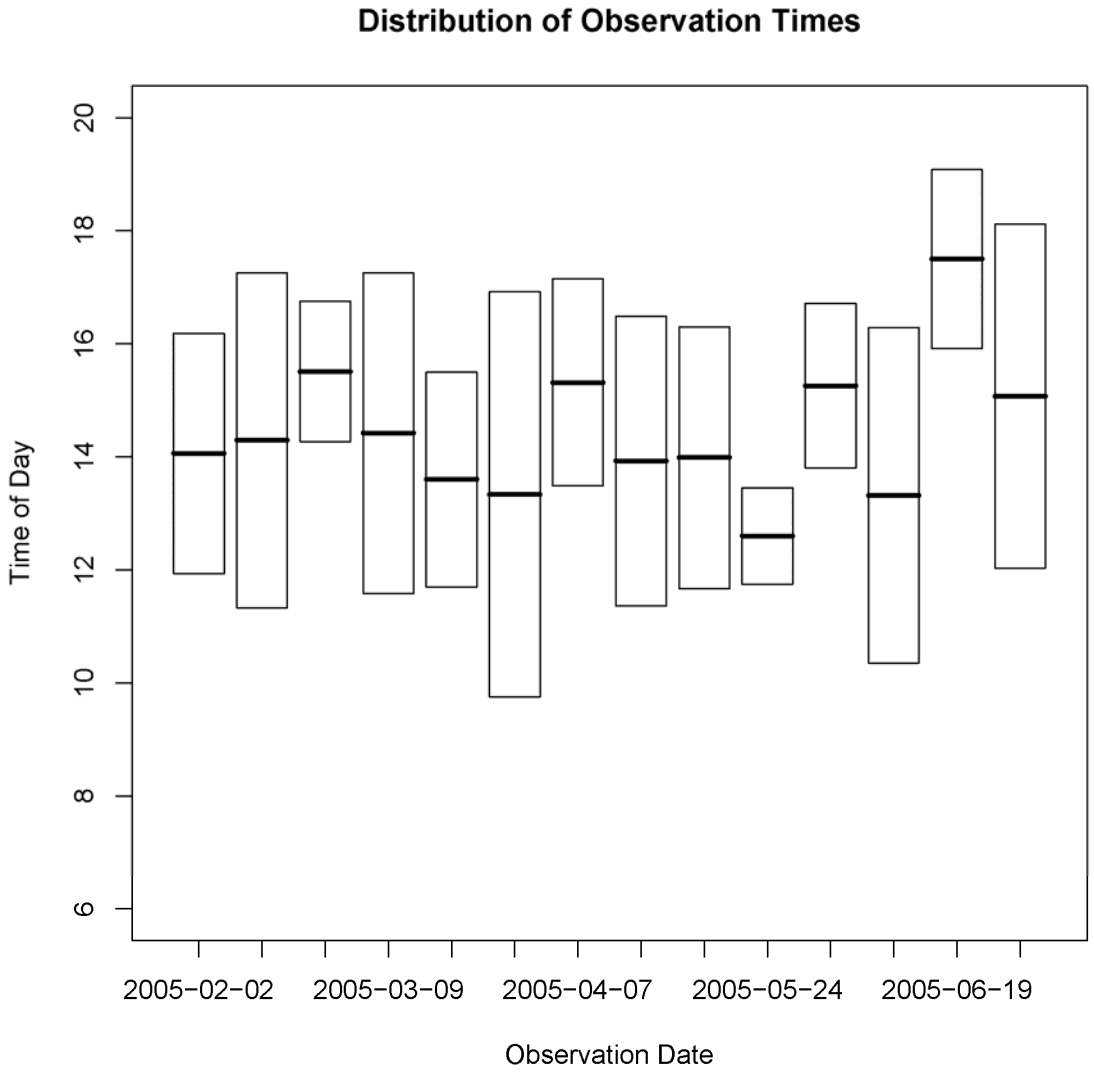

Figure 2.1 Box plot of observation times during 2005. The box extents represent the time when observers were following the whales and the dark lines represent the mid-point of the observation.

Vessel based observation effort in 2005 was significantly greater than in 2003. Fourteen observation periods were conducted between February 2, 2005 and July 1, 2005.. The average observation period lasted 4.64 hours with a minimum of 1.7 hours and maximum of 7.17 hours (Figure 2.1).

GPS track-lines and predation locations (Figure 2.2) clearly demonstrate how these whales used the entire expanse of Hood Canal. Additional locations reported by residents to Orca Network (not shown) present a similar spatial use pattern.

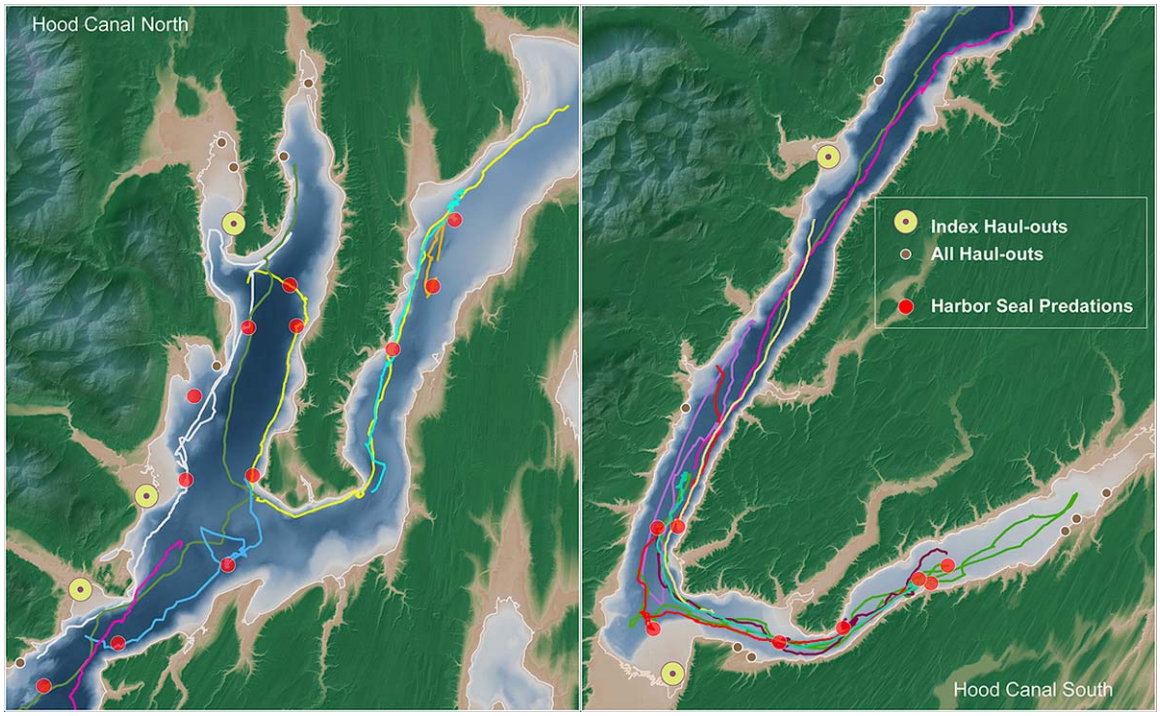

Figure 2.2 Map of North and South Hood Canal showing tracklines from each of the boat based observations and locations of all confirmed harbor seal predations in 2005.

A total of 18 confirmed harbor seal predations were observed during the observation periods. One unsuccessful predation attempt on a California sea lion was also observed, but all other predation events were confirmed as harbor seals. It was not possible to determine the level of individual consumption; therefore a group predation rate was calculated. When adjusted for observation effort, the median consumption rate is 0.329 harbor seals per hour with boot-strapped 97.5 and 2.5 percentiles of 0.465 and 0.215 harbor seals per hour, respectively. The diurnal estimate for total consumption is 758 harbor seals consumed with a boot-strapped confidence interval of 495-1072. The estimate of consumption across all hours is 1358 with boot-strapped confidence interval of 887-1921.

Behaviors observed in Hood Canal appear to be typical of other mammal-eating killer whales. Predation events occurred over deep water, away from any shoreline, and within a few meters of the shoreline in relatively shallow water. The range of behaviors observed was also variable between events. On those occasions when a predation event was relatively short, an oil slick and small remains in the water column were often the only indication of harbor seal presence. However, extended predation events were often characterized by the presence of a seal at the surface. These longer predation events often involved a number of tail slap and ramming attacks on the seal.

Generalized Linear Model

Counts from aerial surveys at five index haul-outs in Hood Canal do not exhibit obvious signs of significant population reduction after either of the killer whale incursions (Figure 2.3). The average count across years from 1996 to 2000 was 684. Huber et al. (2001) have proposed a correction factor for seals in the water of 1.56 for the inland waters of Washington. Thus, the pre-killer whale estimate of seals in Hood Canal is 1068.

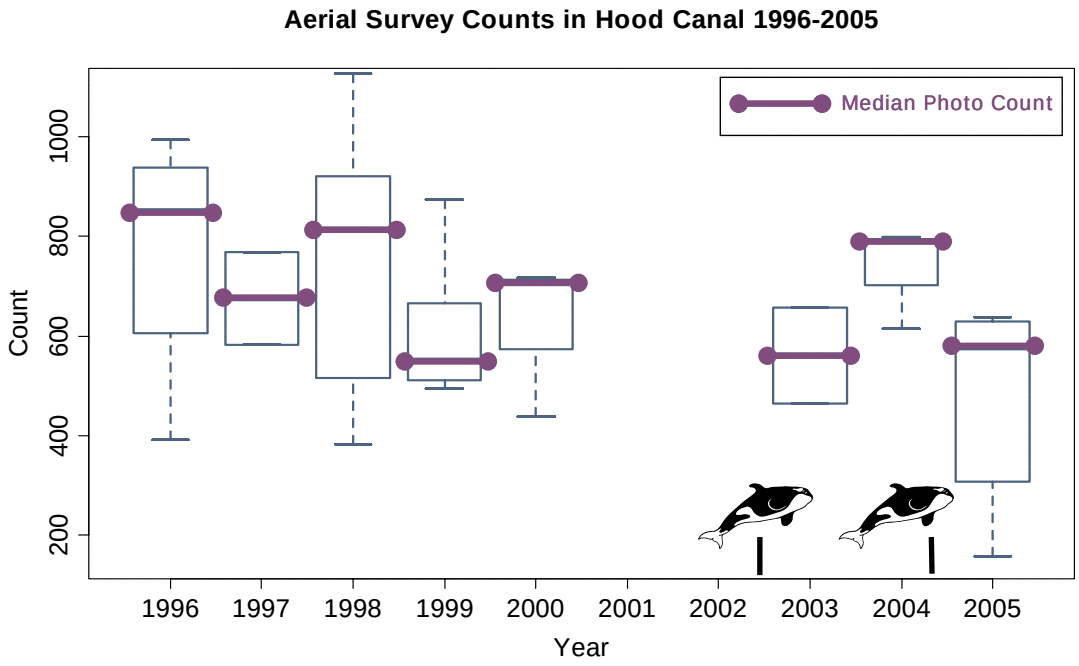

Figure 2.3 Box and whisker plot of aerial survey counts in Hood Canal summed across five index haul-outs for surveys flown between August and November from 1996 to 2004.

AIC values were determined for each GLM representing the four hypotheses (Table 2.2). The 'Treatment + Growth' model was favored with the lowest AIC value of 1070.064. However, the AIC values for the other models resulted in delta values of as little as 1.196 ('Year Model') and as much as 3.233 ('Treatment Model').

Table 2.2 AIC values from four GLMs evaluating hypotheses of harbor seal population response to killer whale predation in Hood Canal, Washington

Model	AIC
No Effect	1072.736
Year Effect	1071.260
Treatment Only	1073.297
Treatment + Growth	1070.064

The treatment and growth coefficients calculated from the GLM under the favored 'Treatment + Growth' model suggest a treatment reduction of 24% (95% CI: +3.5% to -

45.1%) after 2003 and a growth of 49% (95% CI: 0% to 123%) in 2004. Note, for treatment effect and growth, the 95% confidence intervals include 0%.

Bio-energetic Monte Carlo Simulation

Moisture content values were approximately 42 to 51 percent in the two harbor seal carcasses processed (Table 2.3). The yearling harbor seal carcass was recovered from southern Puget Sound and had a mass of 19 kg. The sub-adult animal had a mass of 49 kg and was recovered from Gray's Harbor, Washington.

The values of 2798 kcal/kg for the 49kg sub-adult and 3590 kcal/kg for the 19kg yearling are lower values than reported for ringed seals (Perez 1990) and other pinnipeds (Williams et al. 2004).

Table 2.3 Calorimetric values determined from whole body harbor seal carcasses recovered in Washington State.

Age Class	Mass (kg)	% Moisture	kcal/kg
SubAdult	49 kg	42.6	2798
Yearling	19 kg	50.8	3590

The bio-energetic Monte Carlo simulation for the 2003 event resulted in a median outcome of 997 seals consumed (5th and 95th percentiles: 708, 1435). For the 2005 event, the median outcome determined from the model was 960 (2.5 and 97.5 percentiles: 685, 1383). The distributions of outcomes for both events are strikingly similar (Figure 2.4). The bio-energetic model prediction compares with estimates of 758 and 1358 seals consumed for the diurnal only and all hour assumptions respectively. The estimate from the bio-energetic model falls almost near the midpoint of these two empirical estimates and the all-hour consumption estimate of 1358 is within the 95% confidence range. The daylight only estimate falls just outside the 2.5 percentile.

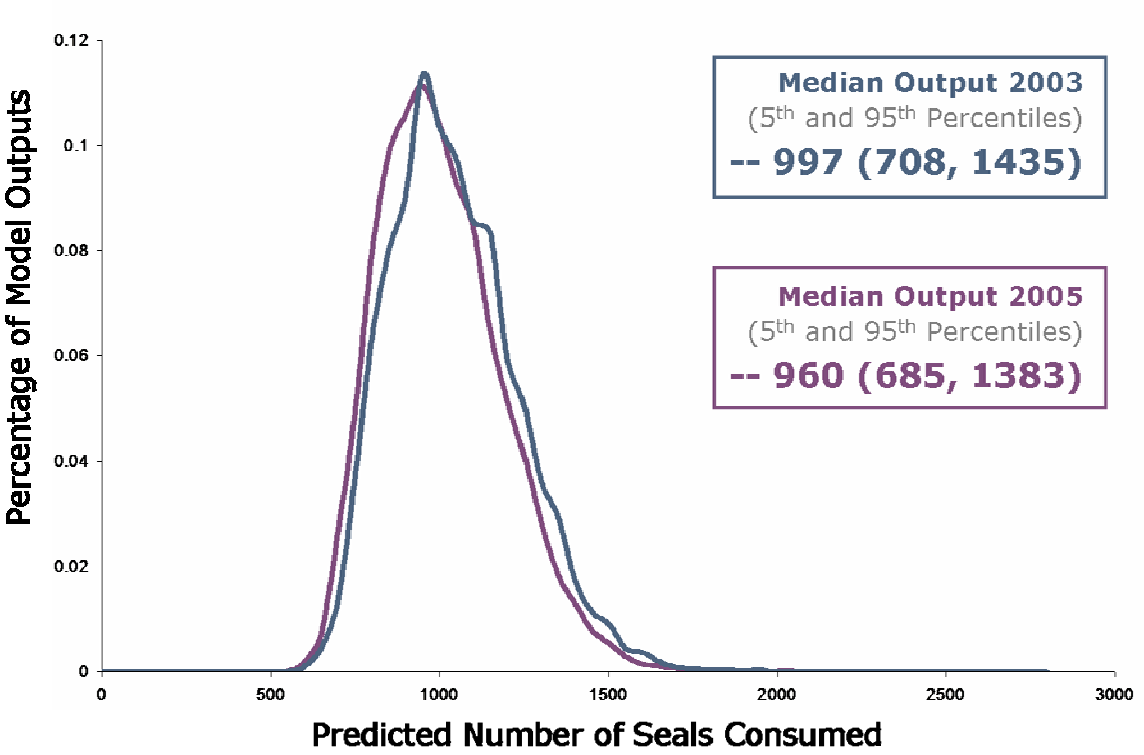

Figure 2.4 Frequency distribution of model outputs from the bio-energetic Monte Carlo simulation for the 2003 and 2005 killer whale incursions.

Discussion

Behavioral Observations

The two extended foraging events by killer whales in Hood Canal differ in many respects from the expected behavior of mammal-eating killer whales. Transient type killer whales are thought to travel in small groups and spend only a few days in one particular area. With stays of 59 and 172 days, the Hood Canal events represent some of the longest reported stays by mammal-eating killer whales in one area. However, the observed behaviors while in Hood Canal were not atypical of those seen in other mammal-eating killer whales. In both 2003 and 2005, the killer whales appear to have used the full expanse of Hood Canal as part of their regular movement and foraging patterns. Neither group exhibited any abnormal behaviors that might be characteristic of a group trapped or lost.

Predation locations were not necessarily associated with harbor seal haul-outs. Harbor seal haul-out locations in Hood Canal are characterized by large, shallow-water, tidal expanses. The physical characteristics of these haul-outs would provide refuge from killer whale predation. Predation locations may better reflect harbor seal foraging locations. The seals would be more vulnerable during foraging activities and some of the predation locations do overlap with confirmed foraging locations from harbor seal movement studies in Hood Canal (WDFW unpublished data).

The estimate of total harbor seal predation during the 2005 event relies on two key assumptions. First, that every predation event occurring during an observation was recorded accurately. Most of the predation activity occurs under water and out of sight of the observer. This limitation is most obvious during shorter predation events when one might only get a fleeting glimpse at the prey animal. During longer events, it was more common to see a harbor seal at the surface and the final consumption was more easily confirmed. To alleviate uncertainty in our predation estimates, we employed a strict protocol for identification of predations. Given other reports of harbor seal predations being very subtle and hard to detect, estimates presented here are probably conservative.

The second assumption critical to the calculation of total harbor seal predation is that the predation rate observed during our observations is representative of the un-sampled period. With the limited observation time and opportunistic nature of the study plan, the robustness of our estimate is lower than would normally be desirable. However, we made a concerted effort to minimize any activity that would contribute any significant bias to the final outcome. It would be reasonable to expect the predation rate of the killer whale group in 2005 to change over the nearly six month presence in Hood Canal due to changes in prey availability or improved knowledge of the area. With only 18 observations, we are unable to examine this possibility. Fortunately, our observation effort is spread relatively even across this period and our estimate of average predation rate would not be overly influenced by any temporal changes.

All predation observations conducted were diurnal and little is known about nocturnal behaviors of mammal-eating killer whales. Baird et al. (in review) present limited data

from time-depth recorders that suggest mammal-eating killer whales have a lower activity level at night compared to daylight hours. Given the uncertainty about circadian changes in foraging rates, we have chosen to present two estimates of total harbor seal consumption. One estimate is extrapolated across just the diurnal period, while the other is across all hours. Activity levels of killer whales are likely influenced by more than just light-level. Actual predation rate likely falls within the bounds of these two estimates.

Bio-Energetic Model

Despite differences in the 2003 and 2005 events (number of days, number of whales, individuals present, age and sex distribution), projected consumption of harbor seals in each year based on the bio-energetic model is strikingly similar. A likely explanation for the model consistency is the importance of prey density and corresponding functional response of the killer whales. As the population of seals in Hood Canal drops due to killer whale predation, so does prey density and prey availability. At some threshold, the cost of finding and catching one more harbor seal in Hood Canal is no longer energetically beneficial and the whales leave. In Hood Canal, this threshold level appears to be a removal of just under 1000 seals and seems to have remained the same across these two events. The longer presence in Hood Canal for the 2005 group is a result of their smaller group size and the absence of large adult males, reducing their combined daily foraging requirements.

Parameters included in the bio-energetic model are reasonable given the current state of knowledge with respect to the ecology and biology of mammal-eating killer whales. By incorporating parameter uncertainty into the model we can better represent our understanding of killer whale bio-energetics and the range of population impacts that could be expected. Validation is a key aspect of any modeling exercise, and observations conducted during the 2005 incursion have provided an opportunity to compare the model predictions with empirical field data. Overall, the bio-energetic model is not inconsistent with empirical estimates determined from field observations. The bio-energetic model and range of parameters used appear to be appropriate predictors of killer whale consumption of harbor seals in Hood Canal.

GLM Analysis of Harbor Seal Counts

The difference between the predicted impact of killer whales on the harbor seal population in Hood Canal and the observed population change is unexpectedly large. The reason for the disparity is unknown at this time. While many of the parameter values and ranges used in the bio-energetic model are not based on empirical data, we feel these values are reasonable based on all current knowledge of killer whale and large mammal bio-energetics. The aerial surveys do exhibit a large amount of variability within and between years. It is not known how much of the variability is due to natural variation and how much is related to sampling variability. A significant factor in this variation may be the influence of human disturbance on haul-out patterns of seals in Hood Canal.

The fact such a rare behavior has happened twice in the same location within two years suggests there may be something especially attractive about Hood Canal. The harbor seal population in Hood Canal is relatively naïve to killer whale predation. Hood Canal is a long and narrow fjord with deep water areas that may provide a situational advantage to the predator. Warmer temperatures and relative quietness of the environment in Hood Canal may also be of importance to killer whales. Any attempt to explain why whales have chosen Hood Canal for these extended stays is mostly speculative at this point. It does, however, seem clear from the bio-energetic models that prey density plays a critical role in determining the timing of departure from Hood Canal.

Chapter 3. Pinniped Scat Genetics: Identification of Sex and Species from Feces Collected for Food Habits Studies

Introduction

Analysis of prey remains found in scats has become the method of choice for investigation of pinniped diets. Scats are pinniped feces deposited on haul-out sites. In most locations, scats can be collected in large numbers with relative ease and minimal disturbance. Interpretation of data from cat analysis, however, does have limitations. Biases associated with recovery and identification of otoliths and bones from some prey species prevent the reliable use of scats for more than generalized characterization of diet (Cottrell et al. 1996; Harvey 1989; Lance et al. 2001; Tollit et al. 2003). Investigation of more detailed aspects of pinniped foraging, such as sexual variation in diet, would require more intensive, potentially intrusive, and expensive methods such as direct observations, enemas, lavaging, or examination of stomachs from harvested individuals.

The potential for significant inter-sexual variation in the foraging ecology of pinnipeds is substantial given there are behavioral and physiological differences that often exist. Strong sexual dimorphism is evident across a number of pinniped species. This, combined with the different energetic demands between males and reproductive females, accounts for differing energetic requirements. Additionally, known cases of seasonal geographic differences between sexes in foraging location and range seem to indicate divergent foraging ecologies in some species (Le Boeuf et al. 2000; Thompson 1989; Thompson et al. 1998).

Scat collection for food habits research is also confounded in some locations where more than one pinniped species share the same haul-out location. Scats are, typically, not reliably distinguished between species and any information from such locations has limited application. Genetic analysis of scat material is a non-invasive technique that would allow individual scats to be classified based on species, sexual or individual identification of the source animal (Kohn and Wayne 1997; Reed et al. 1997).

Genetic scat analysis has been employed in a number of terrestrial mammalian studies (Ernest et al. 2000; Farrell et al. 2000; Fedriani and Kohn 2001; Flagstad et al. 1999; Kohn et al. 1999; Morin et al. 2001; Taberlet and Luikart 1999; Wasser et al. 1997), yet its application to pinniped scat analysis has been limited (Reed et al. 1997). This study presents the development of an efficient and reliable protocol for the extraction of pinniped DNA from scats. While the extracted DNA can provide the basis for a variety of genetic investigations (Kohn and Wayne 1997), here the focus is on amplification of sex specific and species specific markers and their potential use for examination of variation in diet. This methodology, when combined with standard protocols for identification of prey remains from scat, can provide researchers with new insights into the foraging ecology of pinnipeds.

Genetic scat analysis, as discussed here, refers to the use of DNA extracted from fecal samples for investigation of genotypic attributes (e.g. sexual, species or individual identification). Epithelial cells from an animal's intestinal lining are sloughed off during scat deposition and DNA can be extracted from these cells. Various loci (e.g., microsatellites, mitochondrial DNA regions, SRY) can then be amplified through a Polymerase Chain Reaction (PCR) and used to establish sex, species or individual identity. Reed et al. (1997) used genetic scat analysis and microsatellites to assign species of seal to each scat in those areas of the Moray Firth, Scotland where harbor seals and grey seals (*Halichoerus grypus*) share haul-out sites. Of 82 samples collected and analyzed, seal species identification was possible in all but four scats.

In many locations along the Washington and Oregon coastlines, haul-outs are shared by California sea lions and Steller sea lions. Research into the diet of both species in these locations can be confounded because the segregation of the animals on the haul-out is not consistent and the scats cannot be reliably identified to species from visual cues. Therefore, any scats collected from haul-outs with mixed species composition must be discarded or classified as 'generic sea lions,' a category with little interpretive value. This approach ignores the significant possibility that these two species, while using the same haul-out, are utilizing very different areas of the marine ecosystem. Genetic scat

analysis would provide a means for reliable identification of scats to pinniped species so proper segregation and analysis is possible.

Methods

Sample Collection and DNA Extraction

The extraction protocol presented here was adapted from Wasser et al.'s (1997) method for bear scats. A total of 834 scats were collected from five haul-out locations in Hood Canal, Washington in 1999 and 2001. Each scat was collected and stored in individual whirl-packs. Disposable, sterile tongue depressors and latex gloves were used to assist with collection and prevent cross-contamination between scats. Scats were placed on ice while in the field and stored at -20° C as soon as possible.

An extraction buffer, consisting of 500mM Tris-HCL, 16mM EDTA and 100mM NaCl at a pH of 6.0, was added to each scat bag in approximately equal volume to the amount of scat present. Each bag was resealed and the scat-buffer mixture (aka "poop soup") was massaged gently until the scat was thoroughly dissolved. The bag was then set aside to settle for 10-15 minutes. Four 1.5mL tubes were labeled with the scat collection number and four 1mL aliquots were removed from each scat with a pipette and barrier tips. Given the consistency of the "poop soup" it was sometimes necessary to cut a 1/8" section off the pipette tips. Negative control samples containing just the extraction buffer were included in the workflow to monitor any cross-contamination. Tubes were stored in a -20° C freezer. For characterization of diet, remaining scat material was poured through a series of nested sieves and all prey remains were removed and identified in accordance with standard pinniped food habits protocols (Lance et al. 2001).

The use of a liquid medium for separation of sample aliquots differs significantly from other procedures for extraction of DNA from fecal material. The liquid medium not only serves as a buffer, but also provides an efficient means of collecting multiple, independent samples from each scat. The extraction and amplification of multiple tubes in parallel provides further verification of genotypic characteristics in the final analysis (Taberlet and Luikart 1999; Taberlet et al. 1999). The thorough mixing and

incorporation of the buffer and scat also alleviates any concerns that target epithelial cells may not be consistently present throughout each scat. Without incorporation of the buffer, thorough mixing would not be possible without risk of damaging fragile prey remains present in each scat.

The remainder of the genetic extraction involves the use of a Qiagen QiAmp Stool Extraction Kit (Qiagen catalog# 51504). Standard extraction protocol outlined in the stool kit for extraction of human DNA was used with the exception that two 150ul elutions were done at the final stage of the protocol. All extracted samples were stored at -20° C.

Sex Specific Markers

Sex specific loci have been employed in a number a genetic studies for identification of sex. The SRY (sex determining region) and ZFY/X (zinc-finger region) have been used for sexual identification in mammals (Berube and Palsboll 1996; Escorza-Trevino and Dizon 2000; Fedriani and Kohn 2001; Kohn and Wayne 1997; Reed et al. 1997; Wasser et al. 1997). The SRY (Berta et al. 1990) is found on the Y-chromosome and thus only in males, while the ZFY and ZFX loci are homologous regions present on the Y and X chromosome, respectively. Thus, males are identified by the positive amplification of both the SRY and ZFY/X loci, while in females only the ZFY/X loci amplify. A GenBank search revealed a number of primers, in addition to those provided in the literature, designed for amplification of these sex-specific regions. There are, however, three drawbacks to using most of these listed primers. Many were originally designed on the basis of either human or mouse sequences. Successful amplification is possible in pinnipeds, however, the primers and sequence may not match entirely. In the case of degraded DNA or poor PCR conditions, this may result in reduced amplification success. Second, the vast majority of the published primers will amplify both seal and human DNA. This is a considerable problem given the potential for human contamination and the extra time and cost involved in effectively preventing contamination from human sources. Lastly, many of the published primers amplify products greater than 200bp in length. This is only a problem when dealing with highly

degraded and/or fragmented template DNA, as with scats, where small fragment lengths would be ideal (Frantzen et al. 1998).

Given these constraints, new primers were developed based on complete sequence information for the SRY region of harbor seals (GenBank Accession Number: AY424662). The PvSRY2004 primer set (Figure 3.1) was designed with three goals: 1) reliable amplification in pinniped scats, 2) no amplification of human DNA contaminants, and 3) in the case where human DNA did amplify, the ability to detect it as contamination. The primer sequences were designed to match conserved regions in *Zalophus californianus* and *Phoca vitulina* that were differentiated from *Homo sapiens* sequences. The region also included an extra 8-bp that is not present in the human sequence. Successful amplification of the PvSRY2004 region should result in a 91-bp fragment when amplified from pinniped DNA and an 83-bp fragment when amplified from human DNA contaminant. Primer 3 (Rozen and Skaletsky 2000) was used for the final design and evaluation of primer sequences for optimum PCR amplification.

```
                                10         20         30         40         50
                           ....|....| ....|....| ....|....| ....|....| ....|....|
PvSRY2004 Primers                                        T GGGCGGAGAA ATGAGTATTT
Zalophus californianus     TCAGGGG--G CGGGTTTGAG GCAAGGTGCT GGGCGGAGAA ATTAGTATTT
Phoca vitulina             TCGGGGG--G CGGGTTTGAG GCAAGGTGCT GGGCGGAGAA ATGAGTATTT
Homo sapiens               CGGAGAAATG CAAGTTTCAT TACAAAAGTT AA-CGTAACA AAGAATCTGG
Clustal Consensus              *    * *  **** *     *   * *    ** *  * *  * * *

                                60         70         80         90        100
                           ....|....| ....|....| ....|....| ....|....| ....|....|
PvSRY2004 Primers          TAG--->                                      <---CTTA
Zalophus californianus     TAGAAACAAA AGTTACAGCA CCAGAGTGTA GATAATTTTT CGAACGCTTA
Phoca vitulina             TAGAAACAAA AGTTACAGCA CCAGAGTGTA GATAATTTTT CGAACGCTTA
Homo sapiens               TAGAAGTGAG TTTTG----- ---GATAGTA AAATAAGTTT CGAACTCTGG
Clustal Consensus          *****   *    **          **  ***  *  *  *** ***** **

                               110        120        130        140        150
                           ....|....| ....|....| ....|....| ....|....| ....|....|
PvSRY2004 Primers          CACCTTCCAG CTTTGCTACC
Zalophus californianus     CACCTTCCAA CTTTGCTACC CACCCACCCT TTTTTTTTTC CCACCGCTGT
Phoca vitulina             CACCTTCCAG CTTTGCTACC CACCCACCCT TTTTTTCCCC CCACCGCTGT
Homo sapiens               CACCTTTCAA TTTTGTCGC- -ACTCTCCTT GTTTTTGACA ATGCAATCAT
Clustal Consensus          ****** **   ****   *   ** * ** *  *****        *     *
```

Z. californianus sequence excerpted from GenBank AY424650.1
P. vitulina sequence excerpted from GenBank AY424662.1
H. sapiens sequence excerpted from GenBank NM_003140.1

Figure 3.1 Aligned DNA sequences and PCR primers for the newly designed PvSRY2004 region of the Y-chromosome for *Z. californianus*, *P. vitulina* and *H. sapiens*

A GenBank search, unfortunately, did not reveal complete sequence data for the ZFY/X region so that harbor seal specific markers could also be developed as a positive control for reliable identification of female scats. Instead, a microsatellite marker (Pvc63; GenBank Accession Number: L40985) that amplifies reliably in tissue samples collected from seals in Hood Canal was used. Initial screening of the Pvc63 locus indicated an allelic range of 104-108bp.

Non-specific amplification is a common problem when working with DNA extracted from fecal material. Non-specific bands were present on occasion and due to their similarities in size, could have resulted in false sexual identification. In order to prevent ambiguous results, the forward primer of each marker was labeled with different fluorescent labels (PvSRY2004 with FAM and Pv63 with HEX).

Testing and optimizing scat DNA extraction and amplification is often difficult because of the limited opportunity for simultaneous collection of scat samples and higher quality DNA samples (flipper punch, blood, etc) from the same individual. We were able to collect such paired samples on a few occasions during studies of wild harbor seals conducted by the Washington Department of Fish and Wildlife (WDFW) in Puget Sound, Washington, USA. Extractions from these samples were used to confirm appropriate amplification of the primer sets and to optimize PCR conditions for large scale application. Each sample was run on a Molecular Dynamics MegaBACE capillary automated sequencer.

Species Identification Markers

Mitochondrial DNA sequences of Steller's sea lions, California sea lions and harbor seals were extracted from a GenBank search. A multiple alignment was performed with ClustalW 1.8 to determine conserved and differentiated sequence regions. A PinID primer set was designed with four separate primer sequences (Figure 3.2). The reverse primer was designed to correspond with a conserved region across all three species. Three forward primers were designed to match species specific sequence

differentiations. The four primer set will result in amplification of fragments of different size depending on the species of source DNA. Harbor seals will amplify a 170bp region, California sea lions a 129bp region and Steller's sea lions a 134bp region. To further aid in proper species identification, each of the forward primers could be labeled with a different fluorescent label. Testing and optimization of the primer set was done using tissue and scats from each of the species.

```
                                10         20         30         40         50
                           ....|....| ....|....| ....|....| ....|....| ....|....|
PinID_Rev Primer
PinID_Zc Primer                  CTAT TCCCTGACAT GATTAAACTC C--->
PinID_Pv Primer                                      CAATC CCCCTTTCAC TCCTCA--->
PinID_Ej Primer                     CCCTGACAT GACTAGGCCC TC--->
Zalophus californianus     ATTAAACTAT TCCCTGACAT GATTAAACTC CC-----CAC ------ATTC
Phoca vitulina             ATTAAACTAT TCCCTGACGC CCGCCCAATC CCCCTTTCAC TCCTCAATTC
Eumetopia jubata           ATTGGACTAT TCCCTGACAT GACTAGGCCC TC-----CAC ------ATTC
Clustal Consensus          ***  ***** ********            *  *     ***       ****

                                60         70         80         90        100
                           ....|....| ....|....| ....|....| ....|....| ....|....|
PinID_Rev Primer
PinID_Zc Primer
PinID_Pv Primer
PinID_Ej Primer
Zalophus californianus     ATATA-TACC ACTACCCCTA CTGTGCCACC ATAGTATCT- ----------
Phoca vitulina             ATATAATAAT ATCACCT-TA CTGTGCTATC ACAGTATTCA CGCACACTGG
Eumetopia jubata           ATATA-TACC ACTACACCCA CTGTACCACC ACAGTATCTC ----------
Clustal Consensus          ***** **   *  **    * **** * * * * *****

                               110        120        130        140        150
                           ....|....| ....|....| ....|....| ....|....| ....|....|
PinID_Rev Primer
PinID_Zc Primer
PinID_Pv Primer
PinID_Ej Primer
Zalophus californianus     ---------- ---------- ----TTTTTT CCC------- -------CTA
Phoca vitulina             CCTATGTACT TCGTGCATTG CATGTCCCCC CCC-ATCCTC GGACCCCCTA
Eumetopia jubata           ---------- -------TTT CTTTTTTTTT ---------- ----------
Clustal Consensus                                 *

                               160        170        180        190        200
                           ....|....| ....|....| ....|....| ....|....| ....|....|
PinID_Rev Primer                          <---TTGCCCCA TGCATATAAG CACTGTACAT
PinID_Zc Primer
PinID_Pv Primer
PinID_Ej Primer
Zalophus californianus     TGTACATCGT GCAGTTGATG GTTTGCCCCA TGCATATAAG CACTGTACAT
Phoca vitulina             TGTATATCGT GCA-TTAATG GTTTGCCCCA TGCATATAAG CA-TGTACAT
Eumetopia jubata           --TATATCGT TNACTTAATG GCTTGCCCCA TGCATATAAG CA-TGTACAT
Clustal Consensus            ** *****   * ** *** * ******** ********** ** *******
```

Z. californianus sequence excerpted from GenBank
P. vitulina sequence excerpted from GenBank
E. jubata sequence excerpted from GenBank

Figure 3.2 Aligned mtDNA sequences and PCR primers for the newly designed PinID region for Z. californianus, *P. vitulina* and *E. jubatus*

PCR Reactions

Each extracted sample was included in three separate PCR reactions for each primer set (PvSRY2004, Pvc63 and PinID). Muli-plex reactions were tried with the primer sets, but discontinued because there was a significant decrease in reliable amplification and an increase in non-target fragment amplification. Multiple negative and positive controls were included in each PCR plate in addition to negative controls introduced in the extraction procedure. All PCRs were conducted in a separate building and laboratory from the location of the sample processing and DNA extraction to eliminate potential cross-contamination.

Each PCR reaction was 15 µl and included 2 µl of template DNA from the extracted sample. Amplitaq Gold taq polymerase was used at a final concentration of 0.035 U/µl along with a 1x final concentration of Amplitaq Gold PCR buffer. All locus primers were included at a final concentration of 0.3 µM with the exception of the three species diagnostic PinID forward primers which were included at a concentration of 0.2 µM each. Final dNTP concentration was 0.3 mM, 0.6 mM and 0.8 mM respectively for the PvSRY2004, Pvc63 and PinID reactions. Corresponding $MgCl_2$ concentrations were 2.5 mM, 2.75 mM and 2.5 mM.

All reactions were carried out on an MJ Research thermocycler. Thermocycling conditions all involved an initial ten minute denature at 94°C to activate the AmpliTaq Gold. Each reaction also involved a 10-cycle touchdown procedure with an initial annealing temperature five degrees above the target annealing temperature. Each subsequent denaturing, annealing and extension step lasted fifteen seconds. The PCR program was scheduled to run for 35 cycles. A different annealing temperature was used for each locus (PvSRY2004: 56, Pvc63: 52, PinID: 54).

Sex and Species Determination

All samples were initially classified by sex and species based on a strict protocol. Species was determined for those samples that exhibited positive amplification for only

one of the PinID loci. Any sample with product for more than one of the PinID loci was removed from analysis as inconclusive. Sexual identification was determined only for those samples with positive amplification of the Pvc63 marker and the PinID loci specific to *P. vitulina*. Those samples with amplification of the PvSRY2004 fragment were classified as males and those without were classified as females.

A second, less stringent, protocol for classification of sex was employed to include those samples with positive amplification of the PvSRY2004 region and the PinID locus for *P. vitulina*. Since the PvSRY2004 will only amplify in the presence of DNA from males, it is reasonable to conclude these samples are from scats deposited by male seals. The use of these criteria, however, does increase the probability of identifying a male scat compared to a female scat.

Sexual Differences in Diet

Pearson Chi-Square contingency tables were used to test for differences in diet between sexes for each of the top three prey species found in scats in Hood Canal. The top three prey species were determined from a combined frequency of occurrence table across all sites and years for which scats have been collected in Hood Canal (Lance, unpublished data). A 2x2 contingency table was constructed for each of the prey items. The rows represent the presence or absence of the prey species and the columns indicate those scats identified as male or female. This analysis provides initial indications of diet separation between male and female harbor seals in Hood Canal. However, the approach will not account for any species interactions that are likely to exist when comparing complex diets.

Results

For both the PvSRY2004 primer set and PinID primer set, positive amplification was always a correct identification for sex and species when amplified from a known source. All tissue samples resulted in consistent amplification across multiple, independent reactions. Scat samples tended to either work reasonably well, or not at all. Amplification of the mitochondrial DNA PinID marker was more successful than the nuclear DNA Pvc63 and PvSRY2004 markers.

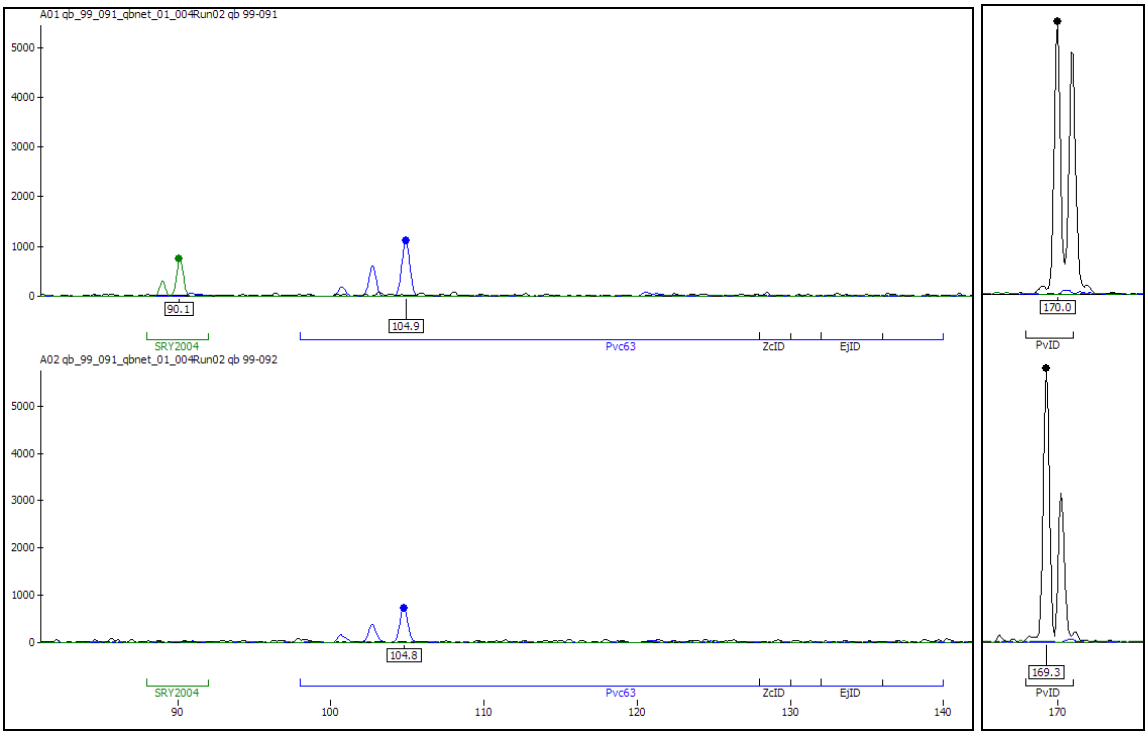

Figure 3.3 Example electropherogram for a male (top) and female (bottom) *P. vitulina* scat. The green peak at 90bp represents a positive amplification of the PvSRY2004 fragment and confirms the male identification. The blue peaks represent the microsatellite Pvc63 and the diagnostic PinID fragment for *P. vitulina* is shown in black at 169bp.

The product size, as measured by the MegaBace Fragment Analysis software, differed slightly from the predicted size (Figure 3.3 and Figure 3.4). This is likely an artifact of the software algorithms used to determine fragment lengths, and not an indication of actual differences in the sequence region amplified. Both the PvSRY2004 and PinID markers often exhibited a secondary peak 1bp larger or smaller in size. The Pvc63 marker exhibited stereotypical microsatellite two base pair stutter patterns.

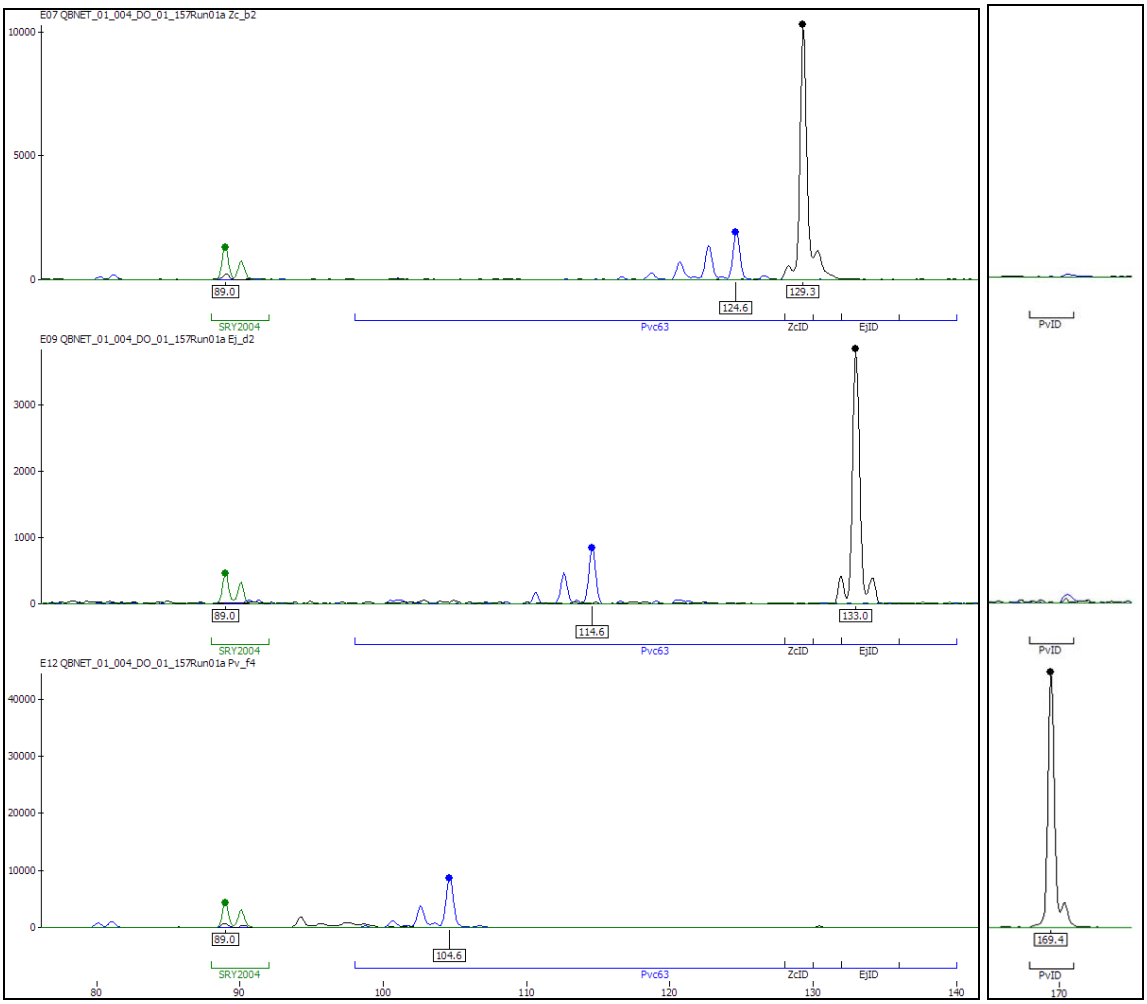

Figure 3.4 Example electropherogram for male scats from *Z. californianus*, *E. jubata* and *P. vitulina*. The green peak at 90bp represents a positive amplification of the PvSRY2004 fragment and confirms the male identification. The blue peaks represent the microsatellite Pvc63 and the diagnostic PinID fragments are shown in black.

Amplification success in scats collected from Hood Canal was greatest for the mtDNA PinID fragment. The success ratio for identification of sex based on the PvSRY2004 and Pvc63 markers differed depending on the level of stringency applied to the classification process. Only 244 of the 831 scats extracted had positive amplification of the Pvc63 and PinID markers (29.4%). Of those 244 scats, 99 (40.5%) were determined to be female and 145 (59.4%) were identified as male. The number scats for which a positive sexual identification was possible increased when those scats exhibiting positive amplification

of the PvSRY2004 and either the Pvc63 or the PinID marker were used. This resulted in an overall success in 370 (44.5%) of the 831 scats extracted.

Frequency of occurrence tables (Lance unpublished data) for all scats collected in Hood Canal between 1998 and 2003 show the top three prey species found in scats to be Pacific hake, Pacific herring and salmon. When compared between males and females, Pacific hake is present in 69% and 66% of the scats respectively and a chi-square comparison found no significant difference ($p=0.268$). There were, however, significant differences in FO values for Pacific herring and salmon. Pacific herring was found in 54% of the female scats, while only 28% of the male scats contained herring ($p<0.001$). The situation was reversed for salmon which was present in only 12% of the female scats compared to 37% for male scats ($p<0.001$).

Discussion

High variability in amplification quality is common among scats (Taberlet et al. 1999). This variability is a result of multiple factors such as degraded template DNA, low concentration of DNA from the extract and large quantities of PCR inhibitors. An adaptation of the multiple tubes approach (Taberlet and Luikart 1999) is suggested to prevent false amplification and the misinterpretation of final results. Ideally, all samples should be amplified in multiple, independent reactions. Funding and time constraints prevented such an approach in this study. This study, however, has focused specifically on the use of presence/absence markers as a means for reducing our susceptibility to errors common in the use of fecal DNA. A large sample size of scats is also a key factor in reducing the impact of any ambiguous amplification, as a higher standard can be set without reducing the number of usable samples, and thereby the statistical power of group comparisons, by a significant percentage. While the overall percentage of scats for which we can confidently assign a sex to is relatively low, the absolute number of scats represents one of the larger sample sizes to date for the examination of sex specific diets in pinnipeds.

Recent terrestrial studies have had greater success amplifying fecal nuclear DNA for genotypic and sexual identification. The reason for the reduced performance in our study

is probably a combination of several factors. A comparison of amplification success rates of nuclear and mitochondrial DNA from scats of captive brown bears on controlled diets was conducted to compare the influence of diet on the amplification of DNA from feces (Murphy et al. 2003). They showed a significantly lower success rate for those scats coming from bears on a salmon diet (26% vs. >60% for other diets). The mechanism for the lower amplification success in scats containing salmon is not clear, especially since mtDNA amplification was not influenced by diet. Murphy et al. (2003) suggest the poor performance may be due to a lower slough rate for intestinal cells as a result of the high lipid content or interference of salmonid by-products with the extraction chemistry. Pacific hake, pacific herring and salmon are the three major components of the harbor seal diet in Hood Canal. All are high lipid prey species that could have a similar impact on amplification success as seen in the Murphy et al. (2003) study.

Amplification success in this study may have also been influenced by the collection protocol, procedural constraints required to maintain the integrity of the prey remains and the time between collection from the haul-out and extraction and amplification. Many of the more successful studies involving the use of fecal DNA employ a desiccation step in the process to impede degradation of the genetic material over time (Nsubuga et al. 2004; Wasser et al. 1997). A concerted effort was made to incorporate the genetic component of this study into the normal practices and procedures of pinniped scat collection and analysis. The high moisture content in pinniped scats and the need to efficiently separate prey remains for identification eliminates the possibility of desiccating the sample for preservation. While there were no strong differences in success between scats collected in 1999 and 2001 (all were extracted in 2002 and amplified in 2004), other studies have demonstrated decreased PCR performance with increased time between collection and extraction/amplification.

The successful development and implementation of the PinID marker set is directly applicable for use in the study of pinniped diets on the West Coast of the United States. California sea lions and Steller sea lions commonly share haul-out locations between

Oregon and British Columbia. Researchers now have a reliable tool for examining differences in diet between these two sea lions species.

The diet differences observed in the initial comparison of male and female scats in Hood Canal are surprising given the minimal sexual dimorphism and generalist diet of harbor seals. Without further details on sex-specific spatial use and foraging behavior, it is difficult to determine the mechanism for this divergence. However, the timing of scat collections overlaps with periods of peak pupping and weaning in harbor seal populations of Hood Canal. The diets may reflect the differences in parental care investment. Salmon are large, mobile prey that may require mor effort and longer trip durations by foraging seals. During this time period, females are likely to be more focused on parental care and to restrict movements to areas near the haul-out. The similar FO values for Pacific hake may reflect an overwhelming availability or preference for this prey species. Further examination of multi-variate relationships and interactions between species present in harbor seal diet should be fully explored.

The study of sexual variation in diet among pinnipeds has been limited mainly to the examination of stomachs from known-sex carcasses (Antonelis et al. 1994; Daneri et al. 2000; Murie and Lavigne 1992). Sexual variation in diet cannot be determined from standard scat analysis protocols. Understanding the role of pinnipeds in various ecosystems is crucial from both a management and conservation perspective. Application of genetic scat analysis for sexual identification can provide researchers with insights into pinniped foraging ecology that were previously unattainable without excessive or highly intrusive efforts. This knowledge is of key importance when applying food habits information from scat to various bio-energetic models to improve conservation measures or understand potential conflicts with commercial fisheries or the recovery of imperiled prey species. The striking inter-sexual variation that characterizes adult anatomy, energy metabolism, and foraging geography in many pinnipeds require that pinniped trophic ecology be understood at the level of gender. As the results from this study show, even species with minimal size differentiation between genders can have observable dietary preferences. We hope the techniques outlined here will be

applied to a variety of pinniped species and potentially lead to a greater understanding of scat analysis and pinniped foraging ecology.

List of References

Adicks, K., J. Ames and J. Haymes. 2004. The Relative Abundance of Adult Salmon in Four Hood Canal Streams During Pinniped Predation Studies Spanning the 1998 - 2001 Return Year. Washington Department of Fish and Wildlife.

Antonelis, G.A., S.R. Melin and Y.A. Bukhtiyarov. 1994. Early Spring Feeding-Habits of Bearded Seals (Erignathus-Barbatus) in the Central Bering Sea, 1981. Arctic 47:74-79.

Baraff, L.S. and T.R. Loughlin. 2000. Trends and Potential Interactions Between Pinnipeds and Fisheries of New England and the U.S. West Coast. Marine Fisheries Review 64.

Beddington, J.R., R.J.H. Beverton and D.M. Lavigne. 1985. Marine mammals and fisheries. George Allen and Unwin, London.

Berta, P., J.R. Hawkins, A.H. Sinclair, A. Taylor, B.L. Griffiths, P.N. Goodfellow and M. Fellous. 1990. Genetic-Evidence Equating Sry and the Testis-Determining Factor. Nature 348:448-450.

Berube, M. and P. Palsboll. 1996. Identification of sex in cetaceans by multiplexing with three ZFX and ZFY specific primers (vol 5, pg 283, 1996). Molecular Ecology 5:602-602.

Brown, R.E., B.E. Wright, S.D. Riemer and J. Laake. 2005. Trends in abundance and current status of harbor seals in oregon: 1977-2003. Marine Mammal Science 21:657-670.

Calambokidis, J., K. Bowman, S. Carter, J. Cubbage, P. Dawson, T. Fleischner, J. Schuett-James, J. Skidmore, B. Taylor and S.G. Herman. 1978. Chlorinated hydrocarbon concentrations and the ecology and behavior of harbor seals in Washington state waters. Cascadia Research Collective.

Calambokidis, J., J.R. Evenson and G.H. Steiger. 1991. Monitoring of harbor seals at Dosewallips state park, Washington. Cascadia Research Collective.

Calambokidis, J. and B. McLaughlin. 1987. Harbor seal populations and their contrbution to fecal coliform pollution in Quilcene Bay, Washington. Cascadia Research Collective, Final Report.

Calambokidis, J., B.D. McLaughlin and G.H. Steiger. 1989. Bacterial contamination related to harbor seals in Puget Sound, Washington. Cascadia Research Collective.

Calambokidis, J., G.H. Steiger, B. McLaughlin and J.R. Evenson. 1990. Harbor seal haul-out habitat and the feasibility of shifting haul-out locations at Dosewallips state park, Washington. Cascadia Research Collective.

Carretta, J.V., K.A. Forney, M.M. Muto, J. Barlow, J. Baker, B. Hanson and M.S. Lowry. 2005. U.S. Pacific Marine Mammal Stock Assessments: 2005. U.S. Department of Commerce, Technical Memorandum NOAA-TM-NMFS-SWFSC-375.

Cottrell, P.E., A.W. Trites and E.H. Miller. 1996. Assessing the use of hard parts in faeces to identify harbour seal prey: Results of captive-feeding trials. Canadian Journal of Zoology-Revue Canadienne De Zoologie 74:875-880.

Daneri, G.A., A.R. Carlini and P.G.K. Rodhouse. 2000. Cephalopod diet of the southern elephant seal, Mirounga leonina, at King George Island, South Shetland Islands. Antarctic Science 12:16-19.

Ernest, H.B., M.C.T. Penedo, B.P. May, M. Syvanen and W.M. Boyce. 2000. Molecular tracking of mountain lions in the Yosemite Valley region in California: genetic analysis using microsatellites and faecal DNA. Molecular Ecology 9:433-441.

Escorza-Trevino, S. and A.E. Dizon. 2000. Phylogeography, intraspecific structure and sex-biased dispersal of Dall's porpoise, Phocoenoides dalli, revealed by mitochondrial and microsatellite DNA analyses. Molecular Ecology 9:1049-1060.

Estes, J.A., M.T. Tinker, T.M. Williams and D.F. Doak. 1998. Killer whale predation on sea otters linking oceanic and nearshore ecosystems. Science 282:473-476.

Farrell, L.E., J. Romant and M.E. Sunquist. 2000. Dietary separation of sympatric carnivores identified by molecular analysis of scats. Molecular Ecology 9:1583-1590.

Fedriani, J.M. and M.H. Kohn. 2001. Genotyping faeces links individuals to their diet. Ecology Letters 4:477-483.

Flagstad, Q., K. Roed, J.E. Stacy and K.S. Jakobsen. 1999. Reliable noninvasive genotyping based on excremental PCR of nuclear DNA purified with a magnetic bead protocol. Molecular Ecology 8:879-883.

Frantzen, M.A.J., J.B. Silk, J.W.H. Ferguson, R.K. Wayne and M.H. Kohn. 1998. Empirical evaluation of preservation methods for faecal DNA. Molecular Ecology 7:1423-1428.

Furness, R.W. 2002. Management implications of interactions between fisheries and sandeel-dependent seabirds and seals in the North Sea. Ices Journal of Marine Science 59:261-269.

Harvey, J.T. 1989. Assessment of Errors Associated with Harbor Seal (Phoca-Vitulina) Fecal Sampling. Journal of Zoology 219:101-111.

Harwood, J. and J.J.D. Greenwood. 1985. Competition between British grey seals and fisheries. Pages 153-169 *in* J. Harwood, J.R. Beddington and D.M. Lavigne, eds. Marine Mammals and Fisheries. George Allen and Unwin, London.

Huber, H.R., S.J. Jeffries, R.F. Brown, R.L. DeLong and G. VanBlaricom. 2001. Correcting aerial survey counts of harbor seals (Phoca vitulina richardsi) in Washington and Oregon. Marine Mammal Science 17:276-293.

Jeffries, S., H. Huber, J. Calambokidis and J. Laake. 2003. Trends and status of harbor seals in Washington state: 1978-1999. Journal of Wildlife Management 67:207-218.

Kohn, M.H. and R.K. Wayne. 1997. Facts from feces revisited. Trends in Ecology & Evolution 12:223-227.

Kohn, M.H., E.C. York, D.A. Kamradt, G. Haugt, R.M. Sauvajot and R.K. Wayne. 1999. Estimating population size by genotyping faeces. Proceedings of the Royal Society of London Series B-Biological Sciences 266:657-663.

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. U.S. Department of Commerce AFSC Processed Report 2001-04.

Le Boeuf, B.J., D.E. Crocker, D.P. Costa, S.B. Blackwell, P.M. Webb and D.S. Houser. 2000. Foraging ecology of northern elephant seals. Ecological Monographs 70:353-382.

Lowry, L.F. and K.J. Frost. 1985. Biological interactions between marine mammals and commercial fisheries in the Bering Sea. Pages 41-61 *in* J.R. Beddington, R.J.H. Beverton and D.M. Lavigne, eds. Marine mammals and fisheries. George Allen and Unwin, London.

Middlemas, S.J., T.R. Barton, J.D. Armstrong and P.M. Thompson. 2006. Functional and aggregative responses of harbour seals to changes in salmonid abundance. Proceedings of the Royal Society of London Series B-Biological Sciences 273:193-198.

Morin, P.A., K.E. Chambers, C. Boesch and L. Vigilant. 2001. Quantitative polymerase chain reaction analysis of DNA from noninvasive samples for accurate microsatellite genotyping of wild chimpanzees (Pan troglodytes verus). Molecular Ecology 10:1835-1844.

Murie, D.J. and D.M. Lavigne. 1992. Growth and Feeding-Habits of Gray Seals (Halichoerus-Grypus) in the Northwestern Gulf of St-Lawrence, Canada. Canadian Journal of Zoology-Revue Canadienne De Zoologie 70:1604-1613.

Murphy, M.A., L.P. Waits and K.C. Kendall. 2003. The influence of diet on faecal DNA amplification and sex identification in brown bears (Ursus arctos). Molecular Ecology 12:2261-2265.

NMFS. 1995. Environmental assessment on protecting winter-run steelhead from predation by California sea lions in the Lake Washington Ship Canal. U.S. Department of Commerce
Washington Department of Fish and Wildlife.

NMFS. 1997a. Environmental assessment on preventing California sea lion foraging and predation on salmonids at Willamette Falls, Oregon. U.S. Department of Commerce.

NMFS. 1997b. Investigation of scientific information on the impacts of California sea lions and Pacific harbor seals on salmonids and on the coastal ecosystems of Washington, Oregon , and California. U.S. Department of Commerce NOAA Technical Memorandum NMFS-NWFSC-28.

NMFS. 1999. Report to Congress: Impacts of California sea lions and Pacific harbor seals on salmonids and West Coast ecosystems. U.S. Department of Commerce, NOAA National Marine Fisheries Service.

Nsubuga, A.M., M.M. Robbins, A.D. Roeder, P.A. Morin, C. Boesch and L. Vigilant. 2004. Factors affecting the amount of genomic DNA extracted from ape faeces and the identification of an improved sample storage method. Molecular Ecology 13:2089-2094.

Olesiuk, P.F., M.A. Bigg and G.M. Ellis. 1990. Recent Trends in the Abundance of Harbor Seals, Phoca-Vitulina, in British-Columbia. Canadian Journal of Fisheries and Aquatic Sciences 47:992-1003.

Perez, M.A. 1990. Review of marine mammal population and prey information for Bering Sea ecosystem studies. National Oceanic and Atmospheric Administration (NOAA) Technical Memorandum National Marine Fisheries Service F/NWC-186.

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.

Rozen, S. and H.J. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. Pages 365-386 *in* S. Krawetz and S. Misener, eds. Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, NJ.

Springer, A.M., J.A. Estes, G.B. van Vliet, T.M. Williams, D.F. Doak, E.M. Danner, K.A. Forney and B. Pfister. 2003. Sequential megafaunal collapse in the North Pacific Ocean: An ongoing legacy of industrial whaling? Proceedings of the National Academy of Sciences of the United States of America 100:12223-12228.

Taberlet, P. and G. Luikart. 1999. Non-invasive genetic sampling and individual identification. Biological Journal of the Linnean Society 68:41-55.

Taberlet, P., L.P. Waits and G. Luikart. 1999. Noninvasive genetic sampling: look before you leap. Trends in Ecology & Evolution 14:323-327.

Thompson, P.M. 1989. Seasonal-Changes in the Distribution and Composition of Common Seal (Phoca-Vitulina) Haul-out Groups. Journal of Zoology 217:281-294.

Thompson, P.M., A. Mackay, D.J. Tollit, S. Enderby and P.S. Hammond. 1998. The influence of body size and sex on the characteristics of harbour seal foraging trips. Canadian Journal of Zoology-Revue Canadienne De Zoologie 76:1044-1053.

Tollit, D.J., M. Wong, A.J. Winship, D.A.S. Rosen and A.W. Trites. 2003. Quantifying errors associated with using prey skeletal structures from fecal samples to determine the diet of Steller's sea lion (Eumetopias jubatus). Marine Mammal Science 19:724-744.

VanBlaricom, G.R., L.R. Gerber and R.L.J. Brownell. 2001. Extinctions of Marine Mammals. Pages 37-69 Encyclopedia of Biodiversity. Academic Press.

Wasser, S.K., C.S. Houston, G.M. Koehler, G.G. Cadd and S.R. Fain. 1997. Techniques for application of faecal DNA methods to field studies of Ursids. Molecular Ecology 6:1091-1097.

WDF, WDW and W.W.T.I. Tribes. 1993. 1992 Washington State Salmon and Steelhead Stock Inventory (SaSI). Washington Department of Fish and Wildlife.

WDFW and P.-N.-P.T. Tribes. 2000. Summer Chum Salmon Conservation Initiative: An Implementation Plan to Recover Summer Chum in the Hood Canal and Strait of Juan de Fuca Region. Washington Department of Fish and Wildlife.

WDFW and P.-N.-P.T. Tribes. 2003. Summer Chum Salmon Conservation Initiative: An Implementation Plan to Recover Summer Chum in the Hood Canal and Strait of Juan de Fuca Region. Washington Department of Fish and Wildlife, Supplemental Report Supplemental Report Number 5.

Williams, T.M., J.A. Estes, D.F. Doak and A.M. Springer. 2004. Killer appetites: Assessing the role of predators in ecological communities. Ecology 85:3373-3384.

Yurk, H. and A.W. Trites. 2000. Experimental attempts to reduce predation by harbor seals on out-migrating juvenile salmonids. Transactions of the American Fisheries Society 129:1360-1366.

Appendix A: Prey Species Present in Scats Classified by Sex

Listed is a table of all scats for which a sex classification was possible using the genetic techniques described in Chapter 3. The sites codes correspond to Quilcene Bay (QB), Dosewallips (DO), Duckabush (DK), Hamma Hamma (HH) and Skokomish (SK). The prey species present in the scat are indicated by the first three letters of the genus followed by the first three letters of the species. When species is not known 'SPP' indicates 'unknown species.' Prey species were identified from all possible diagnostic structures. The diet data is provided by Monique Lance and the Washington Department of Fish and Wildlife (WDFW). These scats are only a subset of all scats collected in Hood Canal between 1998 and 2003 and any characterization of the overall diet of harbor seals in Hood Canal should reference the full dataset available from WDFW.

Sample ID	Sex	Site	Year	Species Present in Scat		
do 99-001	F	DO	1999	CLUPAL		
do 99-002	F	DO	1999	MERPRO		
do 99-018	F	DO	1999	MERPRO	CLUPAL	
do 99-020	F	DO	1999	LOLOPA	MERPRO	CLUPAL
do 99-061	F	DO	1999	SALSPP	CLUSPP	CYMAGG
do 99-080	F	DO	1999	MERPRO		
do 99-087	F	DO	1999	OCTSPP	MERPRO	CLUSPP
do 99-094	F	DO	1999	CYMAGG	CLUPAL	
do 99-104	F	DO	1999	ENGMOR	MERPRO	
do 99-105	F	DO	1999	MERPRO		
do 99-106	F	DO	1999	CLUPAL	MERPRO	
do 99-128	F	DO	1999	MERPRO		
do 99-130	F	DO	1999	MERPRO	SALSPP	CLUPAL
do 99-136	F	DO	1999	CYMAGG	CLUPAL	

Sample ID	Sex	Site	Year	Species Present in Scat		
do 99-140	F	DO	1999	MERPRO	CLUPAL	
do 99-142	F	DO	1999	MERPRO	CYMAGG	
do 99-153	F	DO	1999	MERPRO	CLUPAL	
do 99-166	F	DO	1999	MERPRO	CLUPAL	
do 99-167	F	DO	1999	CLUPAL		
do 99-172	F	DO	1999	CYMAGG	MERPRO	RHAVAC
do 99-173	F	DO	1999	MERPRO		
do 99-176	F	DO	1999	RHAVAC		
do 99-179	F	DO	1999	CLUPAL		
do 99-193	F	DO	1999	MERPRO	CLUPAL	
do 99-003	M	DO	1999	PORNOT	MERPRO	
do 99-010	M	DO	1999	MERPRO		
do 99-015	M	DO	1999	MERPRO		
do 99-019	M	DO	1999	MERPRO	CLUSPP	
do 99-021	M	DO	1999	MERPRO	CLUPAL	
do 99-024	M	DO	1999	MERPRO	CLUSPP	
do 99-027	M	DO	1999	MERPRO	ENGMOR	
do 99-029	M	DO	1999	MERPRO		
do 99-031	M	DO	1999	CLUPAL	MERPRO	
do 99-032	M	DO	1999	CLUPAL	MERPRO	
do 99-033	M	DO	1999	MERPRO	MICPRO	UNKID
do 99-034	M	DO	1999	MERPRO	SALSPP	
do 99-035	M	DO	1999	MERPRO	CLUPAL	AMMHEX
do 99-038	M	DO	1999	MERPRO		
do 99-043	M	DO	1999	MERPRO		
do 99-050	M	DO	1999	MERPRO	CLUSPP	
do 99-064	M	DO	1999	MERPRO		
do 99-071	M	DO	1999	MERPRO		

Sample ID	Sex	Site	Year	Species Present in Scat			
do 99-095	M	DO	1999	MERPRO			
do 99-097	M	DO	1999	MERPRO	ENGMOR		
do 99-102	M	DO	1999	ENGMOR			
do 99-103	M	DO	1999	MERPRO	CLUPAL	ENGMOR	UNKID
do 99-107	M	DO	1999	RAJSPP			
do 99-113	M	DO	1999	MERPRO	CLUPAL	ENGMOR	
do 99-118	M	DO	1999	SALSPP	MERPRO		
do 99-137	M	DO	1999	MERPRO	PORNOT	SALSPP	
do 99-149	M	DO	1999	MERPRO			
do 99-169	M	DO	1999	MERPRO			
do 99-174	M	DO	1999	CLUPAL	MERPRO		
do 99-180	M	DO	1999	CLUPAL	MERPRO		
do 99-181	M	DO	1999	SALSPP	MERPRO		
do 99-184	M	DO	1999	MERPRO	CLUPAL		
do 99-186	M	DO	1999	MERPRO	CLUPAL	SALSPP	
do 99-188	M	DO	1999	SALSPP	CLUSPP		
do 99-199	M	DO	1999	MERPRO	SALSPP	SEBSPP	
do 99-200	M	DO	1999	SALSPP	MERPRO		
do 99-004	M	DO	1999	MERPRO	CLUPAL		
do 99-016	M	DO	1999	MERPRO			
do 99-022	M	DO	1999	MERPRO	CLUSPP	SALSPP	
do 99-030	M	DO	1999	MERPRO	CLUSPP		
do 99-037	M	DO	1999	MERPRO			
do 99-039	M	DO	1999	MERPRO	MICPRO	SALSPP	
do 99-040	M	DO	1999	MERPRO			
do 99-041	M	DO	1999	MERPRO	CLUPAL	SALSPP	
do 99-042	M	DO	1999	MERPRO			
do 99-044	M	DO	1999	MERPRO			

Sample ID	Sex	Site	Year	Species Present in Scat		
do 99-054	M	DO	1999	MERPRO	CLUSPP	
do 99-057	M	DO	1999	MERPRO	CLUSPP	
do 99-058	M	DO	1999	MERPRO	CLUPAL	
do 99-062	M	DO	1999	LOLOPA	MERPRO	
do 99-066	M	DO	1999	SALSPP		
do 99-070	M	DO	1999	MERPRO		
do 99-075	M	DO	1999	MERPRO	CLUPAL	
do 99-076	M	DO	1999	MERPRO		
do 99-079	M	DO	1999	MERPRO	MICPRO	CLUPAL
do 99-086	M	DO	1999	SALSPP		
do 99-091	M	DO	1999	MERPRO		
do 99-092	M	DO	1999	MERPRO	CLUPAL	
do 99-093	M	DO	1999	SALSPP		
do 99-100	M	DO	1999	MERPRO		
do 99-101	M	DO	1999	CLUPAL	MERPRO	
do 99-109	M	DO	1999	MERPRO		
do 99-114	M	DO	1999	CLUPAL	MERPRO	ALOSAP
do 99-115	M	DO	1999	MERPRO		
do 99-122	M	DO	1999	MERPRO		
do 99-124	M	DO	1999	MERPRO	CLUPAL	
do 99-131	M	DO	1999	MERPRO		
do 99-132	M	DO	1999	MERPRO	CLUPAL	
do 99-139	M	DO	1999	MERPRO		
do 99-141	M	DO	1999	SALSPP		
do 99-145	M	DO	1999	MERPRO		
do 99-146	M	DO	1999	MERPRO		
do 99-147	M	DO	1999	SALSPP		
do 99-154	M	DO	1999	CYMAGG		

Sample ID	Sex	Site	Year	Species Present in Scat				
do 99-157	M	DO	1999	CLUPAL	MERPRO	CYMAGG		
do 99-159	M	DO	1999	MERPRO	CLUPAL			
do 99-161	M	DO	1999	MERPRO	CLUPAL			
do 99-171	M	DO	1999	OCTSPP	MERPRO			
do 99-198	M	DO	1999	MICPRO	CYMAGG	CLUPAL	MERPRO	SALSPP
do 99-201	M	DO	1999	MERPRO	CLUPAL			
do 99-025	M	DO	1999	MERPRO				
dk 99-002	F	DK	1999	LOLOPA	MERPRO	MICPRO	LEPARM	CYMAGG
hh 99-010	F	HH	1999	MERPRO	PORNOT			
hh 99-023	F	HH	1999	LOLOPA	CLUPAL			
hh 99-038	F	HH	1999	CLUPAL				
hh 99-008	M	HH	1999	MERPRO	CLUPAL			
hh 99-011	M	HH	1999	SALSPP				
hh 99-019	M	HH	1999	MERPRO				
hh 99-020	M	HH	1999	MERPRO	CLUPAL	SALSPP		
hh 99-030	M	HH	1999	OCTSPP	MERPRO	MICPRO	CLUSPP	
hh 99-036	M	HH	1999	MERPRO	PORNOT			
hh 99-037	M	HH	1999	SALSPP	CLUSPP			
hh 99-009	M	HH	1999	MERPRO	CLUPAL	SALSPP		
hh 99-012	M	HH	1999	SALSPP				
hh 99-013	M	HH	1999	SALSPP	MERPRO			
hh 99-022	M	HH	1999	CLUPAL				
hh 99-002	M	HH	1999	SALSPP				
qb 99-020	F	QB	1999	MERPRO	GASACU			
qb 99-030	F	QB	1999	CLUPAL	MERPRO			
qb 99-033	F	QB	1999	MERPRO	CLUPAL			
qb 99-076	F	QB	1999	OCTSPP	MERPRO	GADSPP		
qb 99-090	F	QB	1999	MERPRO				

Sample ID	Sex	Site	Year	Species Present in Scat			
qb 99-092	F	QB	1999	CLUPAL	ENGMOR	MERPRO	
qb 99-105	F	QB	1999	MERPRO	UNKID		
qb 99-106	F	QB	1999	LEPARM	AMMHEX	CLUPAL	CYMAGG
qb 99-007	M	QB	1999	MERPRO			
qb 99-008	M	QB	1999	MERPRO	GASACU	CLUPAL	
qb 99-017	M	QB	1999	OCTSPP	MERPRO	CLUPAL	
qb 99-022	M	QB	1999	CLUPAL	MERPRO		
qb 99-026	M	QB	1999	MERPRO	PORNOT		
qb 99-034	M	QB	1999	LOLOPA	MERPRO		
qb 99-035	M	QB	1999	MERPRO	CLUPAL		
qb 99-036	M	QB	1999	MERPRO			
qb 99-038	M	QB	1999	SALSPP			
qb 99-039	M	QB	1999	CLUPAL	MERPRO		
qb 99-041	M	QB	1999	SALSPP	MERPRO		
qb 99-047	M	QB	1999	CLUPAL	ENGMOR	SALSPP	
qb 99-055	M	QB	1999	MERPRO			
qb 99-061	M	QB	1999	SALSPP			
qb 99-062	M	QB	1999	SALSPP			
qb 99-063	M	QB	1999	CLUPAL	SALSPP		
qb 99-064	M	QB	1999	CYMAGG	SALSPP		
qb 99-073	M	QB	1999	MERPRO	CLUPAL	ENGMOR	
qb 99-074	M	QB	1999	MERPRO	SALSPP	CLUSPP	
qb 99-091	M	QB	1999	MERPRO	SALSPP		
qb 99-094	M	QB	1999	SALSPP			
qb 99-096	M	QB	1999	SALSPP	MERPRO	CLUPAL	
qb 99-097	M	QB	1999	SALSPP	CLUPAL		
qb 99-098	M	QB	1999	SALSPP			
qb 99-102	M	QB	1999	MERPRO			

Sample ID	Sex	Site	Year	Species Present in Scat					
qb 99-110	M	QB	1999	MERPRO	CLUPAL				
qb 99-112	M	QB	1999	SALSPP	CLUPAL				
qb 99-013	M	QB	1999	CLUPAL					
qb 99-024	M	QB	1999	MERPRO	GASACU				
qb 99-027	M	QB	1999	SALSPP	MERPRO				
qb 99-028	M	QB	1999	MERPRO					
qb 99-040	M	QB	1999	SALSPP	MERPRO				
qb 99-051	M	QB	1999	SALSPP	MERPRO				
qb 99-054	M	QB	1999	MERPRO	CLUPAL				
qb 99-066	M	QB	1999	SALSPP	CLUPAL				
qb 99-070	M	QB	1999	MERPRO	SALSPP				
qb 99-072	M	QB	1999	MERPRO	CLUPAL				
qb 99-095	M	QB	1999	ENGMOR	CLUPAL	MERPRO			
qb 99-104	M	QB	1999	SALSPP	MERPRO	CYMAGG			
qb 99-108	M	QB	1999	MERPRO	CLUPAL	SALSPP	UNKID	GASACU	LYOEXI
sk 99-009	F	SK	1999	CLUPAL	PORNOT				
sk 99-014	F	SK	1999	CLUPAL					
sk 99-019	F	SK	1999	no sample					
sk 99-001	M	SK	1999	SALSPP					
sk 99-003	M	SK	1999	SALSPP					
sk 99-023	M	SK	1999	RAJSPP	MERPRO	CLUSPP			
sk 99-024	M	SK	1999	MERPRO	CLUPAL	ENGMOR			
sk 99-025	M	SK	1999	SALSPP					
sk 99-026	M	SK	1999	CLUPAL					
sk 99-002	M	SK	1999	MERPRO	SALSPP				
sk 99-015	M	SK	1999	CLUPAL					
sk 99-035	M	SK	1999	SALSPP					
do 01-001	F	DO	2001	CLUPAL	GADSPP				

Sample ID	Sex	Site	Year	Species Present in Scat			
do 01-003	F	DO	2001	CLUPAL	GADSPP		
do 01-004	F	DO	2001	CLUPAL	MERPRO		
do 01-006	F	DO	2001	CLUPAL	MERPRO		
do 01-011	F	DO	2001	CLUPAL	RAJSPP	MERPRO	UNKID
do 01-027	F	DO	2001	MICPRO	CYMAGG	AMMHEX	CLUPAL
do 01-032	F	DO	2001	MERPRO	SALSPP		
do 01-040	F	DO	2001	MERPRO	CLUPAL	PORNOT	
do 01-066	F	DO	2001	MERPRO	CLUSPP		
do 01-069	F	DO	2001	CLUPAL	MERPRO	LOLSPP	
do 01-072	F	DO	2001	LOLSPP	MERPRO		
do 01-074	F	DO	2001	CLUPAL			
do 01-076	F	DO	2001	CLUPAL	GADSPP		
do 01-106	F	DO	2001	CLUSPP			
do 01-108	F	DO	2001	CLUPAL	MERPRO	SALSPP	
do 01-125	F	DO	2001	CLUPAL	MERPRO		
do 01-127	F	DO	2001	CLUPAL	MERPRO	SALSPP	UNKID
do 01-129	F	DO	2001	CLUPAL	MERPRO		
do 01-132	F	DO	2001	CLUPAL	MERPRO		
do 01-133	F	DO	2001	CLUSPP	MERPRO	GASACU	
do 01-139	F	DO	2001	CLUPAL	GADSPP		
do 01-140	F	DO	2001	SALSPP	UNKID		
do 01-141	F	DO	2001	CLUPAL	MERPRO	UNKID	
do 01-142	F	DO	2001	CLUPAL	MERPRO		
do 01-146	F	DO	2001	MERPRO	SALSPP		
do 01-149	F	DO	2001	CLUSPP	GADSPP		
do 01-150	F	DO	2001	MERPRO			
do 01-153	F	DO	2001	CLUSPP	MERPRO		
do 01-174	F	DO	2001	MERPRO	CLUSPP		

Sample ID	Sex	Site	Year	Species Present in Scat			
do 01-175	F	DO	2001	CLUPAL	GADSPP		
do 01-178	F	DO	2001	CLUPAL	MERPRO		
do 01-015	M	DO	2001	MERPRO			
do 01-017	M	DO	2001	MERPRO	CLUSPP		
do 01-030	M	DO	2001	MERPRO			
do 01-034	M	DO	2001	MERPRO	CLUSPP		
do 01-035	M	DO	2001	MERPRO			
do 01-036	M	DO	2001	MERPRO			
do 01-037	M	DO	2001	SALSPP			
do 01-038	M	DO	2001	SALSPP			
do 01-046	M	DO	2001	CLUPAL	MERPRO		
do 01-053	M	DO	2001	CLUSPP	MERPRO	MICPRO	
do 01-071	M	DO	2001	CLUPAL	MERPRO		
do 01-082	M	DO	2001	MERPRO			
do 01-087	M	DO	2001	SALSPP	MERPRO		
do 01-092	M	DO	2001	CLUPAL	GADSPP		
do 01-097	M	DO	2001	MERPRO			
do 01-110	M	DO	2001	SALSPP			
do 01-121	M	DO	2001	MERPRO	SALSPP	CLUSPP	
do 01-123	M	DO	2001	CLUPAL	MERPRO		
do 01-128	M	DO	2001	MERPRO			
do 01-134	M	DO	2001	MERPRO	CLUSPP		
do 01-137	M	DO	2001	CLUPAL	GADSPP		
do 01-144	M	DO	2001	CLUPAL	MERPRO	GASACU	UNKID
do 01-152	M	DO	2001	SALSPP	CLUPAL	GADSPP	
do 01-171	M	DO	2001	MERPRO	CLUSPP	SALSPP	
do 01-012	M	DO	2001	CLUPAL	GADSPP		
do 01-013	M	DO	2001	CLUPAL	GADSPP		

Sample ID	Sex	Site	Year	Species Present in Scat			
do 01-025	M	DO	2001	MERPRO			
do 01-026	M	DO	2001	MERPRO			
do 01-029	M	DO	2001	SALSPP			
do 01-031	M	DO	2001	MERPRO			
do 01-041	M	DO	2001	MERPRO	CLUSPP		
do 01-044	M	DO	2001	SALSPP	GADSPP		
do 01-051	M	DO	2001	CLUPAL	MERPRO	GADSPP	MICPRO
do 01-054	M	DO	2001	CLUSPP	GADSPP		
do 01-079	M	DO	2001	CLUPAL	MICPRO	MERPRO	
do 01-083	M	DO	2001	CLUPAL	MERPRO		
do 01-088	M	DO	2001	GADSPP			
do 01-090	M	DO	2001	MERPRO			
do 01-091	M	DO	2001	MERPRO			
do 01-100	M	DO	2001	MERPRO			
do 01-105	M	DO	2001	MERPRO	LOLSPP		
do 01-109	M	DO	2001	CLUPAL			
do 01-111	M	DO	2001	MERPRO	SALSPP		
do 01-112	M	DO	2001	SALSPP			
do 01-115	M	DO	2001	MERPRO			
do 01-117	M	DO	2001	MERPRO	CLUPAL	SALSPP	
do 01-124	M	DO	2001	CLUSPP	MERPRO		
do 01-126	M	DO	2001	MERPRO			
do 01-138	M	DO	2001	CLUPAL	UNKID		
do 01-147	M	DO	2001	SALSPP			
do 01-148	M	DO	2001	CLUPAL	MERPRO	SALSPP	
do 01-151	M	DO	2001	SALSPP			
do 01-154	M	DO	2001	CLUPAL	GADSPP	SALSPP	UNKID
do 01-155	M	DO	2001	MERPRO			

Sample ID	Sex	Site	Year	Species Present in Scat			
do 01-172	M	DO	2001	CLUSPP	MERPRO	GADMAC	
do_01-167	M	DO	2001	MERPRO	SALSPP	CLUSPP	
dk 01-008	F	DK	2001	CLUPAL	MERPRO		
dk 01-012	F	DK	2001	MERPRO	CLUPAL	UNKID	
dk 01-017	F	DK	2001	CYMAGG	CLUPAL		
dk 01-028	F	DK	2001	MERPRO	CLUSPP		
dk 01-040	F	DK	2001	CYMAGG	SALSPP		
dk 01-041	F	DK	2001	CLUPAL			
dk 01-063	F	DK	2001	MERPRO	CLUPAL		
dk 01-064	F	DK	2001	MERPRO	CLUPAL	LOLSPP	
dk 01-109	F	DK	2001	SALSPP	MERPRO	CLUPAL	
dk 01-123	F	DK	2001	SALSPP	UNKID	LOLSPP	
dk 01-011	M	DK	2001	MERPRO			
dk 01-021	M	DK	2001	CLUPAL			
dk 01-105	M	DK	2001	CLUPAL	CYMAGG		
dk 01-126	M	DK	2001	SALSPP			
dk 01-006	M	DK	2001	SALSPP			
dk 01-015	M	DK	2001	MERPRO	SALSPP		
dk 01-036	M	DK	2001	SALSPP			
dk 01-062	M	DK	2001	SALSPP			
dk 01-092	M	DK	2001	MICPRO	CYMAGG	LEPARM	UNKID
dk 01-101	M	DK	2001	GASACU			
qb_generic 01-001	F	QB	2001	CLUPAL	GADSPP		
qb_generic 01-002	F	QB	2001	CLUPAL	MICPRO	CYMAGG	LEPARM
qb_generic 01-007	F	QB	2001	CLUSPP			
qb_generic 01-014	F	QB	2001	CYMAGG	GADSPP	LEPARM	LOLSPP
qb_generic 01-016	F	QB	2001	PORNOT			
qb_generic 01-024	F	QB	2001	CYMAGG	LEPARM	CLUSPP	

Sample ID	Sex	Site	Year	Species Present in Scat			
qb_generic 01-005	M	QB	2001	GADSPP			
qb_generic 01-009	M	QB	2001	no sample			
qb_generic 01-011	M	QB	2001	MERPRO	CLUSPP		
qb_generic 01-012	M	QB	2001	MERPRO	CLUSPP		
qb_generic 01-015	M	QB	2001	MERPRO	CYMAGG	CLUSPP	
qb_generic 01-018	M	QB	2001	SALSPP			
qb_generic 01-020	M	QB	2001	MERPRO	CYMAGG	CLUSPP	LOLOPA
qb_generic 01-021	M	QB	2001	SALSPP			
qb_generic 01-022	M	QB	2001	SALSPP	LEPARM		
qb_generic 01-023	M	QB	2001	SALSPP	LOLSPP		
qb_generic 01-025	M	QB	2001	SALSPP	MERPRO		
qb_generic 01-026	M	QB	2001	MERPRO	MICPRO	LOLSPP	SEBSPP
qb_generic 01-003	M	QB	2001	CLUPAL			
qb_generic 01-006	M	QB	2001	CYMAGG			
qb_generic 01-008	M	QB	2001	MERPRO	SALSPP	CLUSPP	MERPRO
qb_generic 01-010	M	QB	2001	LOLSPP	MERPRO	GADSPP	CLUSPP
qb_generic 01-017	M	QB	2001	CLUPAL	MERPRO		
qb_generic 01-019	M	QB	2001	MERPRO	CYMAGG	SALSPP	
qb_netpen_01-045	F	QB	2001	MERPRO			
qb_netpen 01-002	M	QB	2001	SALSPP			
qb_netpen_01-027	M	QB	2001	SALSPP			
qb_netpen_01-029	M	QB	2001	SALSPP			
qb_netpen_01-031	M	QB	2001	MERPRO			
qb_netpen_01-035	M	QB	2001	GADSPP	CLUPAL		
qb_netpen_01-036	M	QB	2001	MERPRO			
qb_netpen_01-040	M	QB	2001	SALSPP			
qb_netpen_01-042	M	QB	2001	SALSPP			
qb_netpen_01-046	M	QB	2001	LOLSPP	MERPRO	CLUPAL	PLESPP

Sample ID	Sex	Site	Year	Species Present in Scat						
qb_netpen_01-050	M	QB	2001	MERPRO						
qb_netpen_01-051	M	QB	2001	MERPRO	SALSPP					
qb_netpen_01-052	M	QB	2001	GADSPP						
qb_netpen_01-054	M	QB	2001	no sample						
qb_netpen_01-057	M	QB	2001	SALSPP						
qb_netpen_01-058	M	QB	2001	PORNOT	RAJSPP	GADMAC	MERPRO	SALSPP		
qb_netpen_01-061	M	QB	2001	MERPRO	SALSPP	SEBSPP				
qb_netpen_01-062	M	QB	2001	MERPRO	CLUSPP					
qb_netpen_01-064	M	QB	2001	MERPRO						
qb_netpen_01-065	M	QB	2001	SALSPP	GADSPP					
qb_netpen_01-067	M	QB	2001	MERPRO						
qb_netpen_01-068	M	QB	2001	LOLSPP	MERPRO	CLUPAL				
qb_netpen_01-070	M	QB	2001	LOLSPP	RAJSPP	CLUSPP	MERPRO	SALSPP	PLESPP	SEBSPP
qb_netpen_01-072	M	QB	2001	MERPRO	CLUPAL					
qb_netpen_01-073	M	QB	2001	SALSPP						
qb_netpen_01-075	M	QB	2001	RAJSPP	LOLSPP	SALSPP	MICPRO	GADMAC	MERPRO	
qb_netpen_01-038	M	QB	2001	SALSPP	UNKID					
qb_netpen_01-047	M	QB	2001	MERPRO	GADSPP	CYMAGG				
qb_netpen_01-053	M	QB	2001	MERPRO						
qb_netpen_01-056	M	QB	2001	MERPRO	GASACU	GASACU				
qb_netpen_01-059	M	QB	2001	UNKID						
qb_netpen_01-063	M	QB	2001	CYMAGG	CLUSPP	MICPRO	MERPRO			
qb_netpen_01-066	M	QB	2001	MERPRO						
qb_netpen_01-069	M	QB	2001	MERPRO						
qb_netpen_01-071	M	QB	2001	SALSPP	CLUSPP					
qb_netpen_01-074	M	QB	2001	MERPRO	UNKID					
qb_netpen 01-003	M	QB	2001	GADSPP	SALSPP					
qb_oyster 01-024	F	QB	2001	MERPRO						

Sample ID	Sex	Site	Year	Species Present in Scat						
qb_oyster 01-025	F	QB	2001	LEPARM	MICPRO	MERPRO	GASACU			
qb_oyster 01-031	F	QB	2001	MERPRO	CLUPAL					
qb_oyster 01-032	F	QB	2001	MERPRO	CYMAGG	SALSPP				
qb_oyster 01-033	F	QB	2001	MERPRO	ENGMOR	RHAVAC	CLUSPP	LEPARM		
qb_oyster 01-035	F	QB	2001	LEPARM	GADSPP	CYMAGG				
qb_oyster 01-038	F	QB	2001	CLUPAL	MERPRO	GADSPP				
qb_oyster 01-039	F	QB	2001	CLUPAL	MERPRO					
qb_oyster 01-041	F	QB	2001	CLUSPP	MERPRO					
qb_oyster 01-042	F	QB	2001	MERPRO						
qb_oyster_01-005	F	QB	2001	MERPRO	CLUSPP	SALSPP				
qb_oyster_01-018	F	QB	2001	MERPRO	MICPRO					
qb_oyster 01-026	M	QB	2001	LEPARM	MERPRO	SALSPP				
qb_oyster 01-040	M	QB	2001	CLUPAL	MERPRO					
qb_oyster 01-043	M	QB	2001	MERPRO						
qb_oyster_01-019	M	QB	2001	LEPARM	CYMAGG	CLUSPP	SALSPP	UNKID	PLESPP	GADSPP
qb_oyster 01-027	M	QB	2001	GASACU	MERPRO	CLUSPP	SALSPP			
qb_oyster 01-028	M	QB	2001	LEPARM	MERPRO					
qb_oyster 01-030	M	QB	2001	MERPRO						
qb_oyster 01-034	M	QB	2001	MERPRO	CLUSPP	LEPARM				
qb_oyster_01-013	M	QB	2001	SALSPP						

VITA

Joshua Michael London was born in Paris, Texas in 1975 and raised in Tulsa, Oklahoma. He attended Waldo Emerson elementary school, George W. Carver middle school and graduate from Booker T. Washington high school in 1993 before leaving Tulsa to attend the University of Washington in Seattle. Josh graduated from the UW College of Forest Resources with a Bachelor of Science degree in Wildlife Science in 1997. Josh started his graduate research in 1998 and, in 2006, began work for the National Oceanic and Atmospheric Administration, National Marine Fisheries Service as a Wildlife Biologist in the Polar Ecosystems Program at the National Marine Mammal Lab in Seattle, Washington.

Technical Reports

Jeffries, S.J., J.M. London, M.L. Wilson. 1999. Estimates of Harbor Seal Predation on Selected Hood Canal Salmon Runs for Fall 1998. In Pinniped Predation on Salmonid: Preliminary Reports on Field Investigations in Washington, Oregon, and California. National Marine Fisheries Service, NOAA, 7600 Sand Pt. Way NE, Seattle, Washington. 98115.

London J.M., Jeffries S., Calambokidis J. and VanBlaricom, G. 2000. Assessment of Pinniped Predation on Salmonids of Concern in Hood Canal. In The Nature of Hood Canal: Conference Record, Seabeck, Washington. Washington Sea Grant Program. WSG-AS 00-06. Seattle, Washington.

London, J.M. Development of a Protocol for the Extraction of DNA from Pinniped Scats and the Potential Analysis of Individual and Sexual Variation in Diet. In Riemer, S.D. and M.M. Lance. 2001. Pinniped prey identification and quantitative workshop: proceedings of the December 2-4 2000 workshop in Seattle, Washington. National Marine Fisheries Service, NOAA, 7600 Sand Pt. Way NE, Seattle, Washington. 98115.

London, J.M., M.M. Lance and S.J. Jeffries. 2001. Observations of harbor seal predation on Hood Canal salmonids from 1998 to 2000. Final Report, Studies of expanding pinniped populations NOAA Grant No. NA17FX1603, WDFW, PSMFC contract No. 02-15. 20 p.

London, J.M. and G.R. VanBlaricom. 2005. Response of an Isolated Harbor Seal Population to an Extended Foraging Event by Transient-type Killer Whales: Final report to the North Pacific Universities Marine Mammal Research Consortium.

Scientific Presentations

Oral Presentations

London, J.M., S.J. Jeffries, J. Calambokidis, G.R. VanBlaricom. 2000. Assessment of Pinniped Predation on Salmonids of Concern in Hood Canal. The Nature of Hood Canal Conference. Seabeck, Washington. Presented by J.M. London.

London, J.M., S.J.Jeffries, M.L. Wilson, G.R. VanBlaricom. 2000. Foraging Ecology of Harbor Seals in Hood Canal and the Potential Impacts on Threatened Salmonids. 2000. Graduate Student Symposium – University of Washington, School of Aquatic and Fishery Sciences. Seattle, Washington. Presented by J.M. London.

London, J.M., S.J. Jeffries, M.L. Wilson, G.R. VanBlaricom. 2000. Foraging Ecology of Harbor Seals in Hood Canal and the Potential Impacts on Threatened Salmonids. 2000. Washington Cooperative Fish and Wildlife Research Unit Annual Cooperators' Meeting. Seattle, Washington. Presented by J.M. London.

London, J.M., M.M. Lance, P. Bentzen, S.J. Jeffries, G.R. VanBlaricom. 2001. Genetic Scat Analysis: A Practical Approach to Examining Sexual Variation in Diet and the Potential Biases Associated with Pinniped Scats. 14th Biennial Conference on the Biology of Marine Mammals. Vancouver, British Columbia, Canada. Presented by J.M. London.

Jeffries, S.J., J.M. London, M.M. Lance. 2001. A comparison of split-frequency of occurrence and surface predation observation techniques for estimating harbor seal consumption of salmonids in Hood Canal, Washington. 14th Biennial Conference on the Biology of Marine Mammals. Vancouver, British Columbia, Canada. Presented by S.J. Jeffries.

London, J.M., S.J. Jeffries, J.K.B. Ford, P.F. Olesiuk, J. Calambokidis, G.R. VanBlaricom. 2003. Ramifications of an Extended Foraging Event by Eleven Transient Killer Whales on a Population of Harbor Seals in Hood Canal, Washington, USA. 15th Biennial Conference on the Biology of Marine Mammals. Greensboro, North Carolina, USA. Presented by J.M. London.

London, J.M., S.J. Jeffries, J.K.B. Ford, P.F. Olesiuk, J. Calambokidis, G.R. VanBlaricom. 2004. Ramifications of an Extended Foraging Event by Eleven Transient Killer Whales on a Population of Harbor Seals in Hood Canal, Washington, USA. Washington Cooperative Fish and Wildlife Research Unit Annual Cooperators' Meeting. Seattle, Washington, USA. Presented by J.M. London.

London, J.M., S.J. Jeffries, J. Durban, P. Wade, J. Calambokidis, G.R. VanBlaricom. 2005. Case Study: Transient Killer Whales in Hood Canal – Reconciling Theoretical Models with Reality. Special Meeting of the U.S. Marine Mammal Commission on Ecological Role of Killer Whales in the North Pacific. Seattle, Washington, USA. Presented by J.M. London.

Poster Presentations

London, J.M., P. Bentzen, M. Wilson, S.J. Jeffries, G.R. VanBlaricom. 1999. Individual and Sexual Variation in Harbor Seal Food Habits Through the Use of Genetic Scat Analysis. 13th Biennial Conference on the Biology of Marine Mammals. Maui, Hawaii, USA. Presented by J.M. London.

Jeffries, S.J., J.M. London, M.L. Wilson, J. Ames, J. Haymes. 1999. Foraging Ecology of Harbor Seals in Hood Canal and the Potential Impacts on Threatened Salmonid

Stocks. American Society of Mammalogy Conference. Seattle, Washington. Presented by J.M. London.

London, J.M., S.J. Jeffries, M.L. Wilson. 2000. Foraging Ecology of Harbor Seal in Hood Canal and the Potential Impacts on Threatened Salmonid Stocks. Society for Northwestern Vertebrate Biology Conference. Ocean Shores, Washington. Presented by J.M. London.

London, J.M., S.J. Jeffries, M.L. Wilson, G.R. VanBlaricom. 2001. Foraging Ecology of Harbor Seals in Hood Canal and the Potential Impacts on Threatened Summer Chum Stocks. 2001 Puget Sound Research Conference. Bellevue, Washington. Presented by J.M. London

Wilson, M.L., J.M. London, S.J. Jeffries. 2001. Diet of Harbor Seals in Hood Canal During 1998 and 1999. 2001 Puget Sound Research Conference. Bellevue, Washington. Presented by M.L. Wilson.

Awards and Honors

Presentation Awards

Best Student Poster Presentation, Runner-Up Masters Category. 1999. 13th Biennial Conference on the Biology of Marine Mammals. Maui, Hawaii, USA.

Gil Pauley Award for Best Oral Presentation, Runner-up. 2000. Washington Cooperative Fish and Wildlife Research Unit Annual Cooperators' Meeting. Seattle, Washington, USA.

Gil Pauley Award for Best Oral Presentation, Runner-up. 2004. Washington Cooperative Fish and Wildlife Research Unit Annual Cooperators' Meeting. Seattle, Washington, USA.

Best Student Oral Presentation, Ph.D. Category. 2005. 16th Biennial Conference on the Biology of Marine Mammals. San Diego, California, USA.

Service Awards

Unit Leaders' Award: Outstanding Service to the Unit. 2003. Washington Cooperative Fish and Wildlife Research Unit. Seattle, Washington, USA.

Teaching Assistantships

FISH 475: Biology of Marine Mammals. Spring Quarter 2001. University of Washington. Seattle, Washington, USA.

FISH 475: Biology of Marine Mammals. Spring Quarter 2002. University of Washington. Seattle, Washington, USA.