# Causes and Patterns of Harbor Seal (*Phoca vitulina)* Pup Mortality at Smith Island, Washington, 2004-2009

by

Corina L. Leahy

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This Thesis for the Master of Environmental Study Degree

by

Corina L. Leahy

has been approved for

The Evergreen State College

by

---

Gerardo Chin Leo
Member of the Faculty

---

John Calambokidis
Research Biologist
Cascadia Research Collective

---

Martha Henderson
Member of the Faculty

---

Date

# ABSTRACT

Causes and Patterns of Harbor Seal (*Phoca vitulina)*
Pup Mortality at Smith Island, Washington, 2004-2009

Corina L. Leahy

Harbor seals (*Phoca vitulina)* are the most common and widely distributed pinniped in Washington State waters. Their abundance and proximity to land allow many opportunities for examination and necropsy once stranded. Serving as sentinels of marine ecosystem health, stranded animals are useful in detecting environmental contaminant levels and disease in populations. From 2004 to 2009, mortality rates and causes of death of harbor seal (*Phoca vitulina)* pups at Smith Island, a haulout site in North Puget Sound, Washington State, were examined. A total of 16 surveys of this site were conducted during pupping seasons (June through August). Two hundred twelve dead pups were counted, of these 54 were collected for necropsy. Minimum neonatal mortality ranged from 3% to 27%. Neonatal mortality was highest in 2005; half of the total number of dead pups found over the entire study period were collected that year. Infection was the leading primary cause of death in most years. In 2005, 43% of the pups died from an infectious process. In 2006, 2008, and 2009, infection was again the leading cause of death, claiming a total 47% of pups necropsied during those years. The second leading cause of death was malnutrition; other causes of death included prematurity and dystocia. Antibiotic resistant bacteria were isolated from 17 of the 54 pups necropsied. Antibiotic resistant bacterial infections were most prevalent in 2005 and 2009. Bacteria presenting with antibiotic resistance included *Enterococcus, E. coli*, and *Actinomyces*; some of these isolates were found to be resistant to all eight routine antibiotics. As antibiotic resistance becomes more prevalent in marine mammal populations, there could be significant implications for marine ecosystem health. Long term data collection from this site may provide invaluable insights into the potential impacts of contaminants, pathogen introduction, and other perturbations on population recruitment, health and status.

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## ABBREVIATIONS

**ARB**-Antibiotic Resistant Bacteria

**CRC**-Cascadia Research Collective

**MMPA**- Marine Mammal Protection Act

**NMFS**-National Marine Fisheries Service

**NOAA**-National Oceanic and Atmospheric Administration

**SBT**-Sternal Blubber Thickness

**USFWS**-United States Fish and Wildlife Service

**WDFW**-Washington Department of Fish and Wildlife

# INTRODUCTION

## Background and Significance

Marine mammals are an important component of marine ecosystems. They serve as effective sentinels of ecosystem health because of their longevity and extensive fat stores where toxins and contaminants can accumulate (Bossart, 2006; Wells *et al.*, 2004). Harbor seals are a particularly good population to study given that they spend part of their lives in coastal environments and on land, thus making them more accessible for research than many other marine mammals. Unlike other marine mammals, they do not migrate and will remain in one geographic region throughout their life span. This study seeks to understand the factors associated with harbor seal pup mortality in the Puget Sound.

An understanding of causes of mortality in this local marine mammal population can provide essential information for marine mammal management and ecosystem conservation. Monitoring the health of local seal populations is a useful tool for examining the health of the entire Puget Sound ecosystem. Some pathogens that may exist in seal populations have the potential to threaten the health of other marine mammals, such as the endangered orca, or terrestrial animals and scavengers, like the bald eagle. In some instances, seals can even serve as reservoirs of potentially zoonotic pathogens, thus posing a possible health risk to humans. Conversely, in many cases, seals and other marine animals are exposed to pathogens from anthropogenic sources such as agricultural and urban run-off (Bogomolni *et al.*, 2008; Kreuder *et al*., 2003; Miller *et al.*, 2002). The ability to quickly discern subtle changes in seal population health can lead to early detection of potentially devastating environmental disturbances caused by human activity.

While substantial research has been conducted on marine mammals, relatively little is known about the causes of mortality in natural populations. This is due to a variety of limiting factors. Financial cost, man-power, time, and stress to animals can all prohibit or restrict long-term marine mammal population studies. One way to overcome some of these obstacles is by analyzing data from stranded animals. By examining stranded or dead animals, a great deal about mortality, disease, and pathogens in populations can be learned. Stranded animals can be sampled for tissue contaminant levels and can be used to detect pathogens in host populations.

Use of stranded animals is, however, limited. While stranding data may not reflect mortality causes and trends in the entire population, it may provide clues as to what contributing factors or environmental disturbances are significant (Aguilar & Borrell, 1994). This may be particularly true in cases of unusual mortality events or mass strandings. Knowledge of the trends associated with the causes of harbor seal mortality can help determine the relative contribution of disease, malnutrition, or other factors while establishing a baseline of mortalities that can be considered normal for the population. Deviations from these established trends can indicate changes in the environment associated with such disturbances as global climate change, foreign-host pathogen introduction, or anthropogenic disturbances. The ability to distinguish between normal trends and unusual mortality events is essential in marine mammal management and protection. Thus, identification of major causes of mortality can help design effective policies for the management and protection of marine mammals.

**Research Questions, Hypothesis & Approach**

This thesis compares rates and causes of mortality in neonatal harbor seals at Smith Island, Washington over a period of five years. Periodic surveys of the haul-out site were conducted during pupping season from 2004 through 2009. During these surveys, dead pups were collected and necropsies performed when appropriate. I analyzed data collected by Cascadia Research Collective (CRC), from 2004, 2005, 2006, 2008, and 2009 (no surveys were conducted in 2007). I assisted with haulout surveys, necropsies, and data collection in 2009. I also reviewed and analyzed the stranding reports and pathology reports for all years of this study.

During my initial review of this data I noticed that an unusually high number of dead pups were recovered in 2005; more pups were found in that year than in any other year. High numbers of dead pups were found consistently throughout the 2005 pupping season. Determining the potential causes of this marked increase in pup mortality motivated this study. This thesis seeks to answer the following questions:

1) Do primary causes of mortality vary significantly between years?
2) Is there a relationship between pup size (measured by weight, length, and sternal blubber thickness) and cause of mortality?
3) Are there any pathogens or conditions that are consistently prevalent in this population?

My hypotheses are that primary causes of mortality will vary significantly between years; that there is a relationship between cause of mortality and pup size; and that there are pathogens that consistently affect this population.

## Ecology and Biology of Harbor Seals *(Phoca vitulina)*

### Distinguishing Characteristics

Harbor seals are the most common and widely distributed pinniped (fin-footed marine mammals) in Washington waters. They are easily distinguishable from other seals by their round, dog-like faces and short snouts. As true (earless) seals, they have no external ear flaps. Their bodies and flippers are short. Their pelage (coat) patterns are variable, most harbor seals exhibit a lightly colored base with dark spots; some individuals will exhibit a reverse pattern of white spots over a mostly black or dark brown coat. Seals with intermediate coloration are common as well (WDFW, 2009).

Harbor seals are recognizable on land as they tend to resemble bananas when hauled out, elevating their head and rear flippers (NMFS, 2009). Their hind flippers lack flexibility resulting in undulating or scooting movements while on shore. Harbor seals are small in comparison to other seals. Average length and weight can vary between populations. In the Pacific Northwest, adult harbor seals range from 1.2 to 1.9 m in length with an average weight of 80kg. Females are usually smaller than males. Pups typically weigh 7 to 8 kg at birth (WDFW, 2009; NMFS, 2009).

## Distribution, Movements, & Population Patterns

Harbor seals occur over a latitudinal range from about 30°N to 80°N in the eastern Atlantic region and about 28°N to 62°N in the eastern Pacific region. They have the widest distribution and occur in more different habitats than any other pinniped (Burns, 2008). While total global population estimates vary, eastern Pacific harbor seal populations are fairly abundant. In waters from Alaska to California, the total population is estimated to be near 350,000 individuals (Carretta *et al.*, 2007). In Washington state, harbor seals are abundant and by some reports, near carrying capacity. In 1999, it was determined that the inland Washington stock totaled an estimated 14,612 seals. At that time, the total Coastal Washington/Oregon population was estimated to be at 24,732 seals (Jeffries *et al.*, 2003).

Harbor seals are generally non-migratory, staying in the same area throughout the year to feed and breed. Local movements within a region can be associated with such factors as weather, season, tides, food availability, and reproduction (Bigg, 1981). Harbor seals have also displayed strong fidelity for particular haulout sites (Pitcher & McAllister, 1981).

For management purposes within Washington State, two distinct stock populations are recognized. The first, Washington inland stock, includes those seals found in all inland waters of the state (including Puget Sound, Hood Canal, and the Strait of Juan de Fuca out to Cape Flattery). The second consists of seals found along the Washington/Oregon coastal regions (Boveng, 1988). This thesis will focus on one site in the inland Washington region.

The inland waters region of Washington is of particular interest as the health of the Puget Sound has drastically declined. High levels of environmental contaminants have been found in the resident orca population, shellfish are frequently not safe to eat due to toxin levels, and storm water runoff are just a few of the threats to the health of this region. Monitoring seal populations within this region can provide valuable insight into the state of the Sound.

## Foraging, Breeding Habitat & Haulouts

Harbor seals are generalists and will typically forage on easily available and abundant foods (Burns, 2008). Their diet may vary with seasonal availability of prey but primarily consists of several species of fish and cephalopods. Harbor seals generally feed in shallow waters close to shore and as mentioned, may exhibit strong site fidelity.

Harbor seals breed in both coastal and insular waters. Seals give birth in rookeries on shore. During breeding season, herds of seals can be found at these sites, hauled out in large groups with no apparent social structure.

Pinnipeds haul out on land for thermal regulation, predator avoidance, social interaction, and parturition. Harbor seals may haul out on rocks, beaches, glacial ice, reefs or islands. In Washington, harbor seals typically haul out on beaches with limited access, remote islands or remote beaches (Figure 1). In Puget Sound, seals will also frequently haul out on log booms or man-made floats.

*Figure 1. Harbor seals hauled out at Smith Island, Washington.*

## Reproduction & Mortality

Female harbor seals reach sexual maturity at ages of 3 to 4 years; physical maturity is reached at the age of 6 to 7 years. Males reach sexual maturity at 4 to 5 years and physical maturity at 7 to 9 years (Burns, 2008). The maximum lifespan of a harbor seal is between 30-35 years, although individuals rarely live this long in the wild. Females tend to live longer than males yet mortality for both sexes is highest during the first few months after birth (Riedman, 1990). Individuals are reproductively active throughout their lives with females typically giving birth to one pup per year, although twinning has been observed (Burns, 2008). The gestation period is approximately 10.5 months.

In most regions, Washington included, pups are born on land. Pupping season varies throughout populations. Even within Washington, pupping season varies by location, but tends to occur fairly consistently at each site across seasons. In inland Washington waters, pupping season starts in late June and lasts through early September (WDFW, 2009). Pups are nursed for approximately 4 to 6 weeks and can triple their weight by the time they are weaned. These fat reserves are useful as the pups learn to forage on their own.

Several factors can adversely affect survival, often with varying effects on different age classes. In young or first time mothers, the risk of abortion or stillbirth is higher. As these females are typically smaller, they may in turn give birth to smaller offspring thus increasing vulnerability to injury or hypothermia (Geraci & Lounsbery, 2008). Starvation or malnutrition can also lead to death, particularly in dependent young pups, immunocompromised individuals, or older animals. Trauma may lead to mortality in seal populations, especially at crowded haulout sites where the density of animals can increase the chances of accidental trauma, particularly to small pups. Pathogens are another significant source of mortality. Parasitic, bacterial, viral, and fungal infections can all contribute to seal death. Seal pups are also more likely to fall victim to predation, as they are often left alone and vulnerable on shore.

In Washington State, transient orcas, eagles, gulls, and coyotes all prey on harbor seals (Lambourn, *et al*., 2010; Steiger *et al*., 1989). A number of anthropogenic factors can also affect harbor seal survival. Environmental contaminants (Calambokidis *et al*., 1985; Ross *et al*., 1993), pollution and debris, and fisheries interactions can all pose threats to harbor seal health.

## METHODS

### Study Site Background

Smith Island was chosen as the study site because it is subject to relatively low levels of human disturbance. This is due to the fact that the island is part of the San Juan Islands National Wildlife Refuge; access to the island is restricted, requiring a federal permit from the United States Fish and Wildlife Service (USFWS). Smith Island is a small, rocky island located within the eastern Strait of Juan de Fuca (48°19'N, 122°50'W) (Figure 2). It is connected to the even smaller Minor Island, by a spit, which is visible during low tide. The rocky substrate is an ideal haulout site for seals as the pups are well camouflaged on the beach, easily blending in with the rocks. The site is also a nesting habitat for gulls and bald eagles. For this study, both Smith and Minor Islands were surveyed. For simplicity, the study site will collectively be referred to as Smith Island.

### Permits and Survey Date Selection

Survey permits were obtained from USFWS. Annual survey dates were chosen to coincide with the peak of pupping season at Smith Island (late June through early August) and precede molting. Attempts were made to schedule multiple survey dates each year, approximately two weeks apart. Dates were selected during low tide and were subject to personnel and vessel availability as well as weather. Due to these constraints, no surveys were conducted in 2007 and only one survey was conducted in 2008. Subsequently, this study includes data collected from surveys conducted in 2004, 2005, 2006, 2008, and 2009, (Table 1). Although surveys were conducted prior to 2004, the sampling effort varied greatly. Thus, surveys prior to 2004 are not included in this study.

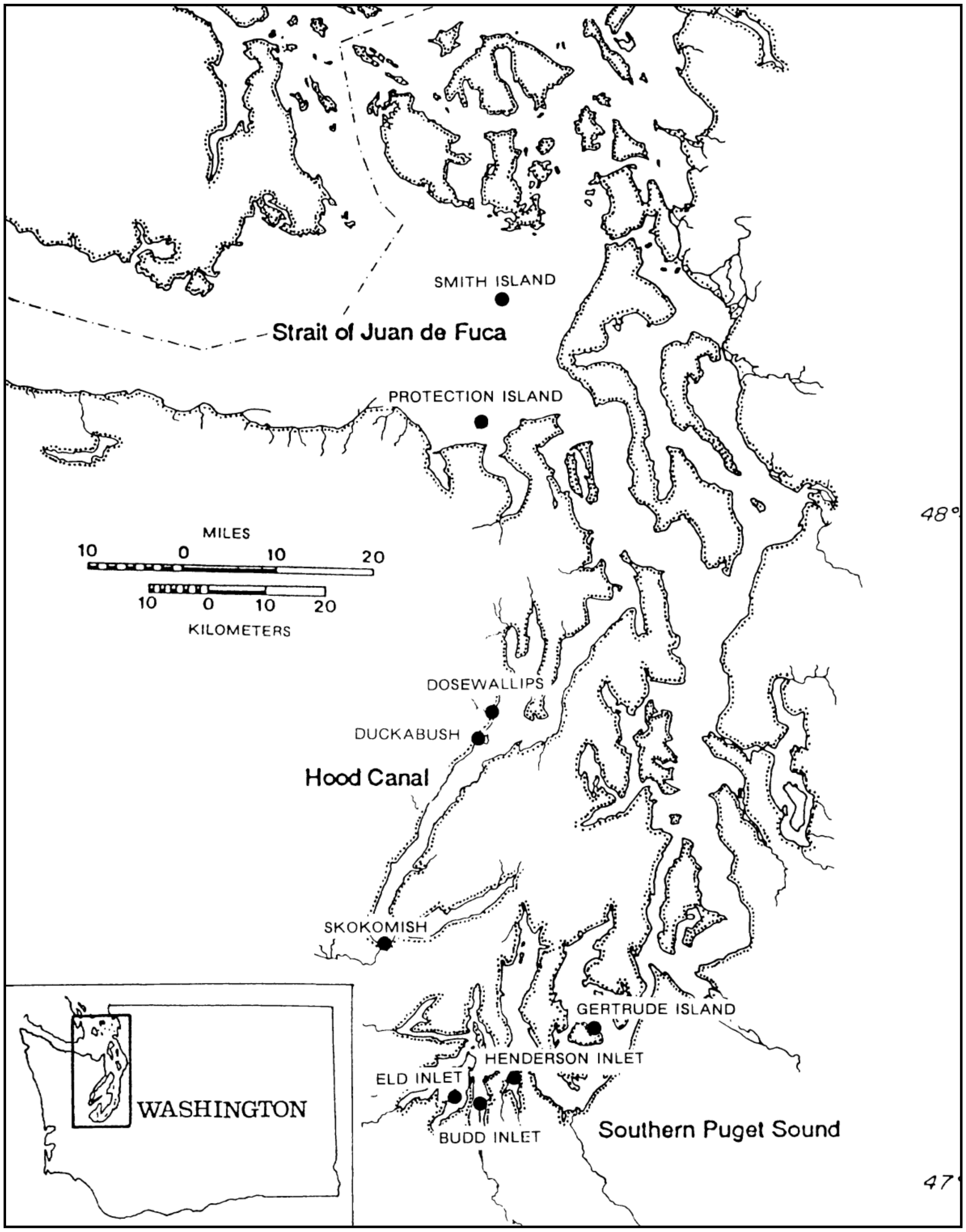


*Figure 2.Map of harbor seal haulout sites within inland Washington waters. (Steiger et al., 1989)*

Table 1. Survey dates during each year of study period.

| Year | | | | |
|---|---|---|---|---|
| 2004 | 2005 | 2006 | 2008 | 2009 |
| 6-Jun | 7-Jul | 6-Jul | 5-Aug | 8-Jul |
| 21-Jun | 10-Jul | 12-Jul | | 22-Jul |
| 30-Jun | 13-Jul | | | 20-Aug |
| 9-Jul | 25-Jul | | | |
| 15-Jul | 7-Aug | | | |

**Survey Procedures**

The haulout site was reached by small boat. In order to determine count estimates of seals hauled out, photographs were taken during approach; it is necessary to take photographs on approach as all healthy seals will head into the water when disturbed. Sighting estimates of adults and pups hauled out and in water were recorded. Surveyors then landed near the eastern end of Minor Island. Surveys were conducted by a team of at least two people. When sufficient personnel were available, effort was divided by two teams, with one team taking the north side of the islands, the other taking the south. All dead seals were recorded and photographed. If biologists were not able to get to a carcass, due to location or proximity to nesting gulls, photos were taken and the carcass was included in count. Once counted, carcasses were marked to prevent duplicate counts on future surveys.

**Data and Sample Collection**

Cascadia Research Collective is a member of the National Ocean and Atmospheric Administration's (NOAA) National Marine Fisheries Service (NMFS) Stranding Network and collects Level A data on all marine mammal strandings they respond to. Level A data includes date and time of stranding, species, age class, sex, weight, standard length (measured from tip of snout to tail), and evidence of human interaction. In addition to these measurements, blubber thickness and axillary girth was measured for all carcasses found when feasible. Blubber thickness was measured ventrally, at the sternum. Axillary girth was measured around the animal at the axilla of the front flippers.

Only relatively fresh, minimally scavenged carcasses were collected for complete necropsy. NMFS utilizes a number system to code decomposition levels of marine mammal carcasses, described as follows: Code 1: live animal; Code 2: fresh dead; Code 3: moderate decomposition; Code 4: advanced decomposition, and Code 5: mummified or skeletal remains. Carcasses collected for necropsy were typically Code 2.

**Necropsy and sample collection**

Whole carcasses collected for necropsy were taken back to the WDFW game farm in Lakewood for complete exam and necropsy per established protocols (Pugliares, *et al.,* 2007) by CRC or WDFW staff. A detailed external exam was conducted on all pups prior to necropsy. Any external findings were documented and photographed as necessary. All major organ systems were examined and sampled during internal exam (Table 2). Photographs were also taken of internal findings. Samples were collected for frozen and formalin preservation.

Additional samples such as wound tissue, wound cultures, fluid cultures, or fluid samples were collected as needed. Samples were submitted to Dr. Stephen Raverty, veterinary pathologist, at Animal Health Center, British Columbia, Ministry of Agriculture and Lands, for gross and microscopic exam. Immunohistochemistry, serology, and PCR assays were undertaken as appropriate.

Site selection, survey protocols, sample collection and necropsy methods, were established by Cascadia Research Collective prior to this study. The following methods were chosen by the author for this study.

Table 2. Routinely sampled tissues & corresponding preservation type

| | Preservation | |
|---|---|---|
| Tissue Sample | Frozen | Formalin |
| blubber | x | x |
| brain | x | x |
| colon | x | x |
| eye | x | x |
| gallbladder | x | x |
| glands | x | x |
| heart | x | x |
| intestine | x | x |
| kidney | x | x |
| liver | x | x |
| lung | x | x |
| lymph nodes | x | x |
| muscle | x | x |
| pancreas | x | x |
| reproductive tract | x | x |
| skin | x | x |
| spleen | x | x |
| stomach | x | x |
| tonsil | x | x |
| trachea | x | x |
| urinary bladder | x | x |
| blood | x | |
| feces | x | |
| pericardial fluid | x | |
| serum | x | |
| stomach contents | x | |
| urine | x | |
| vitreous humor | x | |

## Development of Methods and Study Design

### Measurement Selection

Length, sternal blubber thickness, axillary girth, and weight were recorded for many pups in this study. However, due to scavenging, it was often not possible to record accurate axillary girth. Blubber thickness, weight and length were chosen for comparison as they were consistently measured on most pups.

### Calculating Percent Mortality

Annual minimum mortality rates were calculated using the total number of dead pups found as a percentage of the pups born (Calambokidis *et al*., 1985; Lambourn, *et al.*, 2010). The total number of pups born was calculated using the highest number of pups seen at one time, plus the total number of dead pups found. Total pup count estimates were determined from examination of aerial and vessel-based survey photos provided by CRC and WDFW.

Total count estimates were conducted both by CRC and WDFW. For CRC estimates, photos were taken from boat before landing on the island for surveys. Data from WDFW estimates was collected via aerial surveys. These surveys were typically conducted in August when seal congregations are highest as it is the end of the pupping season and beginning of the adult molting season. Total WDFW counts appear to be more accurate as all parts of the island could be seen at the same time; CRC staff could only photograph whatever side of the island they were approaching from. Due to the higher count reliability and consistency in survey dates, WDFW photos were chosen to use for seal count estimates.

### Determining Cause of Mortality

Primary cause of mortality is defined as the condition most likely to have caused the animal's death based on all information provided (Colegrove *et al*., 2005).

In order to determine the cause of mortality for necropsied pups, the following were examined:

- initial stranding data and photographs
- necropsy photos and notes
- histopathology reports

The most significant factor in determining cause of mortality was the total results of the pathology report. Additional information from initial stranding response forms and necropsy notes was used as needed for clarification.

# RESULTS

## General Findings

From 2004 through 2009, a total of 212 dead pups were found during 16 total surveys (Figure 3). Fifty-four of these pups were found to be suitable for necropsy and were collected (Table 3). Higher numbers of dead pups were found in late July and early August, towards the end of pupping season. This is to be expected most weak or abandoned pups will have expired by the end of the season. A total of 27 premature (lanugo) pups were found over the course of the study; of these, nine were necropsied. Pups were classified as premature if they had 50% or greater coverage of lanugo (the soft white coat covering newborn seals; in harbor seals, it is typically shed in utero) on their bodies. Length was obtained for 146 of the total 212 dead pups. The mean standard length for premature pups was 79 cm ($n$=17, SD=4.5); the mean standard length for full-term pups was 81 cm ($n$=129, SD=5.8). The sex of each pup, if determined (often precluded by scavenging), was recorded (Table 4). A total of 66 female pups and 77 male pups were found over the entire study period. Scavenging and pup location precluded sex determination for the remaining 69 pups.

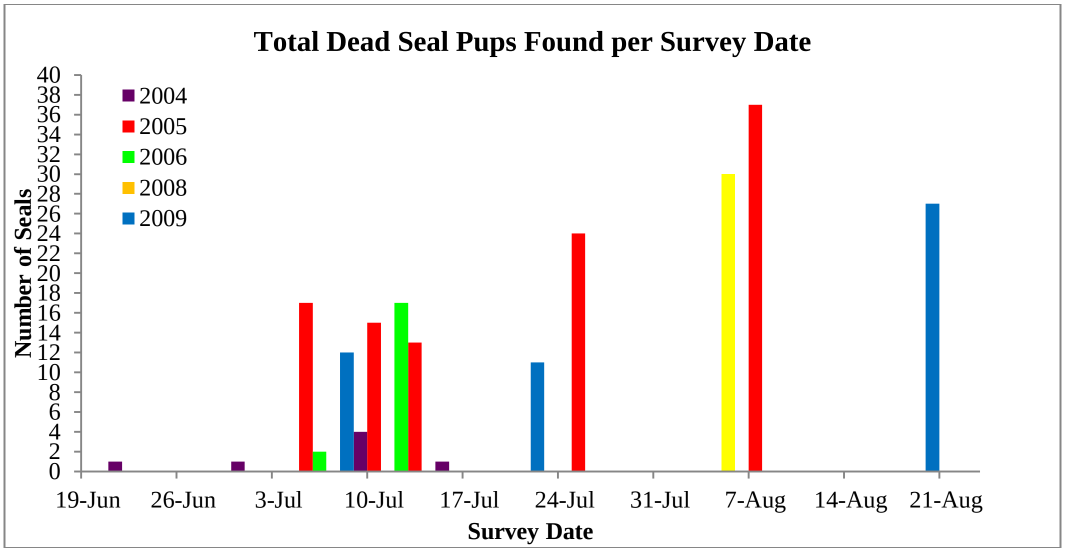


*Figure 3.Total dead pups found for all survey date for all years, (grouped by week).*

Table 3. Number of pups found & necropsied by survey date.

| Date | Number of Pups | |
|---|---|---|
| | Found | Necropsied |
| 6-Jun-04 | 0 | 0 |
| 21-Jun-04 | 1 | 0 |
| 30-Jun-04 | 1 | 0 |
| 9-Jul-04 | 4 | 2 |
| 15-Jul-04 | 1 | 0 |
| 5-Jul-05 | 17 | 10 |
| 10-Jul-05 | 15 | 5 |
| 13-Jul-05 | 13 | 2 |
| 25-Jul-05 | 24 | 12 |
| 7-Aug-05 | 37 | 6 |
| 6-Jul-06 | 2 | 0 |
| 12-Jul-06 | 17 | 3 |
| 5-Aug-08 | 30 | 6 |
| 8-Jul-09 | 12 | 4 |
| 22-Jul-09 | 11 | 4 |
| 20-Aug-09 | 27 | 0 |
| Total | 212 | 54 |

Table 4. Number of female & male pups found per year.

| | Number of Pups | | |
|---|---|---|---|
| Year | Female | Male | Unable to Determine |
| 2004 | 3 | 2 | 2 |
| 2005 | 45 | 37 | 24 |
| 2006 | 2 | 10 | 7 |
| 2008 | 7 | 6 | 17 |
| 2009 | 9 | 22 | 19 |
| Total | 66 | 77 | 69 |

**Causes of Mortality**

Primary cause of mortality was determined for 40 of the 54 seals necropsied. The causes of mortality were divided into four major categories:

- Stillborn/dystocia- stillborn pups and those that died as a result of a traumatic birth
- Malnutrition/emaciation- pups which died as a result of starvation
- Infectious-pups which died as a result of bacterial, viral, or parasitic infection
- Unable to determine- pups for which necropsy revealed no significant findings, or those for which post-mortem decomposition or carcass condition hindered histopathological examination

In some cases, secondary or contributing causes were also determined (Table 5).

Table 5. Primary (P) and contributing (C) causes of mortality by year.

| Cause of Mortality | 2004 P | 2004 C | 2005 P | 2005 C | 2006 P | 2006 C | 2008 P | 2008 C | 2009 P | 2009 C | Total P | Total C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Stillborn/dystocia | 2 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 8 | 0 |
| Malnutrition/emaciation | 0 | 0 | 8 | 9 | 1 | 2 | 0 | 1 | 0 | 4 | 9 | 16 |
| Infectious | 0 | 0 | 15 | 1 | 2 | 0 | 1 | 0 | 5 | 0 | 23 | 1 |
| Unable to determine | 0 | 0 | 8 | 0 | 0 | 0 | 5 | 0 | 1 | 0 | 14 | 0 |
| Total examined | 2 | | 35 | | 3 | | 6 | | 8 | | 54 | 17 |

While there was variability in causes of death between years, primary causes of mortality did not vary significantly (Pearson $\chi^2$=12.598, df=8, p-value=0.126) between years. (This analysis only included three categories: stillborn/dystocia, malnutrition/emaciation, and infectious as the category unable to determine is not an actual cause of death). In 2004, both seals necropsied were found to be stillborn. This was evidenced by the fact that one pup was found still in the fetal sac and the lungs for both pups sank in formalin; indicating that the pups had not respired. In 2005, most pups (43%) died as a result of infection. Malnutrition was the second highest (23%) primary cause of mortality that year. In many cases (26%), malnutrition was a secondary cause of mortality. In 2006, only three pups were necropsied; of these, two died from infection as the primary cause and malnutrition as the secondary cause. The third pup died as a result of malnutrition. In 2008, all but one of the animals collected were too decomposed to determine cause of mortality. That seal was found to have died from infection. In 2009, infection was again the leading cause of mortality (63%) while malnutrition was the leading contributing or secondary cause (50%). Cause of mortality was determined for 6 of the 9 lanugo pups. Two died as a result of stillbirth/dystocia. Two died from malnutrition. The remaining two died from an infectious primary cause.

**Pup Size (Measured by Length, Weight, & Sternal Blubber Thickness (SBT)) In Relation to Cause of Mortality**

Pup length did not vary significantly by cause of mortality (ANOVA, p=0.285). Pup weight did vary significantly with cause of mortality (ANOVA, p<0.001). Sternal blubber thickness also varied significantly (ANOVA, p=0.001). This is not surprising as one of the causes of mortality was malnutrition/emaciation; these pups would have had lower weights and blubber thickness.

**Percent Mortality**

Estimated minimum percent mortality for pups (calculated as described in methods) was determined for 2004 through 2008 (Table 6). Seal count survey data from 2009 were not yet available.

These mortality rates are only minimum estimates as some carcasses were likely scavenged or washed away with the tide. The highest rate of mortality (27%) occurred in 2005. This was markedly higher than in all other years, where mortality ranged from only 3% to 16%. While there did not appear to be a great increase in the total number of all seals that year, surveys did show a higher number of pups in 2005 than in other study years. Calculated birth rate was also significantly higher (46%) that year; birth rates in the remaining survey years ranged from 18% to 22%.

**Other Significant Findings**

**Antibiotic Resistant Bacteria**

Antibiotic resistant bacteria (ARB) isolates were found in 30% (n=16) of the pups necropsied. Bacterium exhibiting antibiotic resistance included *Enterococcus sp.* (Table 7), *E.coli* (hemolytic and non-hemolytic) (Tables 8 & 9), *and Actinomyces sp.* (Table10). Several isolates were resistant to multiple antibiotics. This is an unexpected finding in a wild population and is most likely caused by fresh water run-off (Bogomolni *et al.*, 2008; Stoddard *et al.*, 2005). As newborns with underdeveloped immune systems, these pups may have been inherently more susceptible to bacterial infections and it is not known what effects ARB may have on the population as a whole.

**Phocid herpes virus (PhHV-1)**

Four of the pups collected for necropsy tested positive for phocine herpes virus (PhHV-1). Three of the pups were found in 2005, one was found in 2009. In all cases, the cause of mortality was infection. All of these pups exhibited simultaneous bacterial infections. In such cases, the bacteria could be the primary pathogen; a latent PhHV-1 infection occurring as a result of an already stressed immune system (Gulland *et al.*, 1997).

***Streptococcus canis***

In 2005, two seals were found with *Streptococcus canis* infections. *Streptococcus canis* is an opportunistic pathogen in canids; exposure typically involves close contact with an infected individual, so this is an unusual finding in harbour seals. In both cases, this isolate was considered significant and likely represented an environmental source of infection via the umbilicus. Both seals presenting with *Streptococcus canis* died as a result of infection; one from omphalophlebitis (infection of the umbilical vein), and one from omphalitis (infection of the umbilicus) with subsequent peritonitis. *Salmonella typhimurium* was also found in one of these seals.

***Salmonella typhimurium***

*Salmonella typhimurium* was isolated from four seals in 2005. In all four cases, this finding was significant as this bacteria contributed to mortality. This is also an unusual finding. This disproportionate number of pups found with *Salmonella typhimurium* is concerning as it may have represented some sort of environmental exposure such as untreated sewage runoff, farm runoff or exposure from other wildlife. A source has yet to be determined. *Salmonella typhimurium* was not seen in other years of this study. As with most bacterial and viral pathogens, suppressed immunity caused by malnutrition or stress may have led to an increased susceptibility to infection.

Table 6. Annual seal counts, calculated birth rates & minimum percent mortality.

| | Highest Seal Count | | Highest Live Pup Count | | Dead Pup Count | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Year (A) | Date (B) | Number (not including pups) (C) | Date (D) | Number of pups (E) | Total found (F) | Found after highest pup count date (G) | Minimum pups born (E + G) (H) | Birth rate (H/C) (I) | Minimum neonatal mortality (F/H) (J) | Apparent pups not dying (H-F) (K) |
| 2004 | 16-Aug | 1303 | 11-Aug | 245 | 7 | 0 | 245 | 19% | 3% | 238 |
| 2005 | 4-Aug | 844 | 4-Aug | 352 | 106 | 37 | 389 | 46% | 27% | 283 |
| 2006 | 11-Aug | 1163 | 10-Aug | 205 | 19 | 0 | 205 | 18% | 9% | 186 |
| 2008 | 15-Aug | 893 | 15-Aug | 192 | 30 | 0 | 192 | 22% | 16% | 162 |

(counts were not yet available for 2009)

Table 7. Pups with antibiotic resistant *Entercoccus sp.* isolates (r=resistance)

| | CRC stranding number | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Antibiotic | 586 | 587 | 588 | 594 | 612 | 614 | 622 | 630 | 885 | 942 | 951 | 952 |
| enrofloxacin | r | r | r | r | r | r | r | | | r | | |
| erythromcyin | | | r | r | r | r | | | r | r | | |
| gentamycin | | | r | r | r | r | r | | | | | |
| lincomycin | r | r | r | r | r | r | r | r | r | r | | r |
| penicillin | | | r | r | r | r | | | r | | | |
| sulfamethoxazole/ trimethroprim | r | r | r | r | r | r | r | r | r | r | r | |
| tetracycline | | | | r | r | r | r | | r | r | | |
| florfenicol | | | r | | r | | r | | | | | |

Table 8. Pups with antibiotic resistant *E. coli* (non-hemolytic) isolates (r=resistance)

| | CRC Stranding Number | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Antibiotic | 587 | 594 | 612 | 614 | 630 | 885 | 930 | 940 | 942 | 948 | 951 | 952 |
| enrofloxacin | | | | | r | | | | | | | |
| excenel | | | | | | | | | | | | |
| gentamycin | | | | | | | | | | | | |
| neomycin | r | | | | | | r | r | r | r | r | r |
| ampicillin-sulbactum | r | | | | r | | | | | | | r |
| sulfamethoxazole/ trimethroprim | | r | r | r | | r | | | | | | r |
| tetracycline | r | | | | r | r | | | | | | r |
| florfenicol | | | | | | | | | | | | |

Table 9. Pups with antibiotic resistant *E. coli* (hemolytic) isolates (r = resistance)

| Antibiotic | CRC stranding number | | |
|---|---|---|---|
| | 588 | 594 | 630 |
| enrofloxacin | | | |
| excenel | | | |
| gentamycin | | | |
| neomycin | | | |
| ampicillin-sulbactum | | | |
| sulfamethoxazole/trimethroprim | r | r | |
| tetracycline | | | r |
| florfenicol | | | |

Table 10. Pups with antibiotic resistant *Actinomyces sp.* isolates ( r= resistance)

| Antibiotic | CRC stranding number | | |
|---|---|---|---|
| | 614 | 622 | 636 |
| enrofloxacin | r | r | |
| erythromcyin | r | r | |
| gentamycin | r | r | |
| lincomycin | r | r | |
| penicillin | r | r | |
| sulfamethoxazole/trimethroprim | r | r | r |
| tetracycline | r | r | |
| florfenicol | r | r | |

# DISCUSSION

## Standard Length and Prematurity

The total number (n=27) of premature pups found during this study appears to be lower than previously recorded at this site (Steiger *et al*., 1989*).* The mean standard length (79cm) for premature pups appears to be slightly higher than previously determined (69cm) while the length (81cm) of term pups is quite similar to that found previously (84cm). This could be explained by multiple variables. The previous study examined multiple sites in one year; pups from other sites could have influenced the standard length calculations. Also, in at least two cases, relatively large lanugo pups were found in my study. In such a small sample size, this can influence the mean standard length. However, both studies did report a difference in length between premature and term pups as would be expected.

## Causes of Mortality

The primary and secondary causes of mortality found are consistent with those previously reported at Smith Island. Prematurity, stillbirth/dystocia, and malnutrition were prevalent in this study as well as previous studies. However, more pups appear to have succumbed to infection over this study period than previously observed (Steiger, *et al.*, 1989).

We were only able to necropsy more than 5 pups in three of the study years (2005, 2008, and 2009). The highest numbers of pups were collected and necropsied in 2005 and 2009; infection was the leading cause of mortality in both of these years. Based on these findings, it is likely that infection could be a prominent cause of death in most years.

Only four categories were used for cause of death in this study. I chose these categories to simplify the analysis. Categories could be broken down further to suit the purposes of further studies. For example, infection could be broken down into viral, bacterial, and parasitic sources. Other classification schemes (Bogomolni *et al.*, 2010) exist but it seems that more general classifications, such as those used in this thesis help simplify comparisons between studies, particularly when examining mortality across a variety of marine mammals.

### Pup Size In Relation to Cause of Mortality

The relationship between pup size and cause of mortality is to be expected. As sternal blubber thickness and weight are indicators of pup health, it is not surprising that pups with lower weights and inadequate blubber thickness succumbed to emaciation. What is more difficult to discern is when emaciation is the primary cause of death rather than a contributing factor. One of the most difficult tasks of this study was determining this. In some cases where malnutrition and infection contributed to mortality, it is difficult to say which came first. Malnutrition can lead to weakened immunity which can in turn lead to infection. Conversely, animals weakened by infection can become anorexic, thus succumbing to malnutrition. As newborns, pups are under stress and have relatively low immunity regardless. It is easy to assume that one single factor is responsible for pup mortality; but in essence, all life events cumulatively lead to mortality.

### Percent Mortality

The significant increase in neonatal mortality in 2005 appears to correlate with a dramatic increase in birth rate. This is an interesting finding and suggests that the increase in pup mortality that year was likely a function of the higher number of pups born that year. It is likely that pup mortality at Smith Island is highly variable and dependent on a number of factors, such as prey resources and maternal age at pupping. Previous work (Calambokidis *et al*., 1985) has found smaller size and higher mortality in pups born to young primiparous females.

### Other Significant Findings

While some level of antibiotic resistance is expected, this level of multi-antibiotic resistant bacteria in a wild population is unusual and concerning. Alarmingly, antibiotic resistance has also been found in other marine mammals and seabirds along the Northeastern United States (Rose *et al*., 2009). The source of this resistance is not clear but *Enterococcus* and *E. coli* are pathogens of human concern as well. Antibiotic resistance in seals is likely contributed to the prevalence of antibiotic use in humans and agricultural animals. As antibiotic resistance becomes more prevalent in marine mammal populations, there could be significant implications for marine ecosystem health.

Studies have demonstrated that PhHV-1 appears to be endemic in Pacific harbor seal populations but that fatal infections usually only occur in neonates (Goldstein *et al*., 2003; Gulland *et al*., 1997; Harder *et al*., 1997). It is unknown what percentage of the population carries PhHV-1. Infected seal pups from my study were also included in a recent review (Himworth *et al*., 2010) of all PhHV-1 cases presenting in British Columbia, Canada and Washington state; most of these seals presented with other simultaneous infections. This is true of the PhHV-1 seals in my study. This makes it difficult to distinguish what role PhHV-1 may have played in the mortality of these seals. PhHV-1 could have predisposed these seals to other virulent infections; conversely, these infections could have weakened pup immunity and subsequently increased the pathogenicity of PhHV-1. Regardless, PhHV-1 appears to be at least a contributing factor in the loss of these pups.

It is unclear what may have contributed to the prevalence of *Streptococcus canis* and *Salmonella typhimurium* in this population. *Streptococcus canis* might be expected in a coastal population where dogs and other canids could come in contact with hauled out seals; this is not the case in an isolated location such as Smith Island. *Salmonella typhimurium* is another unusual finding. The most likely source of these bacteria is coastal or untreated sewage runoff. No point source was ever determined and *Salmonella typhimurium* has not been isolated in this population during any year other than 2005.

**Study Limitations**

When interpreting the results of this study, it is important to keep in mind a number of limitations. This study examined one age class at one over several years. Causes of death may vary by age class or site. All surveys in this study occurred during pupping season; causes of mortality in pups may vary as they grow and are no longer subject to such a densely packed haulout environment. A certain sampling bias also exists as only fresh carcasses were collected. A number of carcasses were lost to decomposition and scavenging or were washed out with the tide. Also, as only dead animals were sampled, the effect some of these pathogens may have on live animals is unknown.

The results of this study indicate the annual variability in percent pup mortality, causes of pup mortality, and birth rate in one population of harbor seals within Washington inland waters. Care should be used in extrapolating these results to other populations within the region or other geographical areas.

## CONCLUSION & SUGGESTIONS FOR FURTHER RESEARCH

This study has found that at Smith Island, primary causes of death in harbor seal pups did not vary significantly between years. The findings did demonstrate a relationship between pup size and cause of mortality. Infectious disease, malnutrition, stillbirth, and prematurity were all common causes of death in pups at this site. Common pathogens, such as *Enterococcus* and *E. coli* were found in this population as well as some more unusual findings such as PhHV-1, *Streptococcus* canis, *Salmonella typhimurium*, and high levels of ARB.

As this study only examined one age class, future studies should include multiple age-classes and multiple sites if possible. WDFW and CRC have conducted studies at other sites; continuing this work is essential. Future work should also focus on comparing studies between regions throughout the United States, as well as comparisons between other marine mammal species. Standardizing the way data is managed within the various stranding networks would help facilitate this.

This thesis demonstrates the wealth of information that can be learned about a population through the use of stranding data. Perhaps the most important finding of this study is the detection of common pathogens and overall patterns in mortality. Long-term population monitoring is important to help understand population dynamics and support critical management decisions. Continuing the current work of CRC, WDFW, and other agencies is integral to our understanding of local marine mammal populations. When we understand what is "normal" in a population, we are better equipped to quickly identify population shifts and disturbances. Studies such as this also provide a window into the health of the entire ecosystem. As we become more aware of anthropogenic sources of degradation in the marine environment, we must be able to quantify the effects on both animals and the ecosystem as a whole. Long-term data collection from this site may provide invaluable insights into the potential impacts of contaminants, pathogen introduction, and other perturbations on population recruitment, health and status. As seals are sentinels of environmental health, monitoring their health is a tool for monitoring the health of the Puget Sound and all its inhabitants.

As part of this ecosystem, our well-being is dependent on its sustainability. We must therefore actively participate in the monitoring and conservation of its resources. Thus, a critical complement to long-term population studies such as this would be a comprehensive social analysis on the anthropogenic disturbances to Puget Sound ecosystem health. Assessing current perceptions on the health of Puget Sound and how the public relates to the marine ecosystem is vital to public education and the eventual minimalization of anthropogenic effects.

Increased prevalence of antibiotic resistant bacteria, emerging pathogens, and environmental contaminants affect the health of the entire ecosystem. Monitoring water quality, mitigating urban and agricultural run-off, and proper use of anti-microbial therapy are all essential to maintaining a healthy marine ecosystem. If such measures are not taken, the environmental effects will be even more severe than they are now, potentially lethal for many species. A combination of multiple long-term studies of several marine species, public education, and effective environmental policy are needed if we are to conserve and sustain our marine resources.

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