# CAUSES AND PATTERNS OF HARBOR SEAL (*PHOCA VITULINA*) PUP MORTALITY AT SMITH ISLAND, WASHINGTON, 2004–2010

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ABSTRACT—Harbor Seals (*Phoca vitulina*) are the most common and widely distributed pinniped in Washington State coastal waters. Serving as sentinels of marine ecosystem health, stranded animals are useful in detecting environmental disease and contaminant levels. From 2004 to 2010, we examined mortality rates and causes of death of Harbor Seal pups at Smith Island, a principal haulout site in the eastern Strait of Juan de Fuca, Washington, conducting 21 site surveys during the pupping season (June through August). We documented and externally examined 245 dead pups and of these, 72 were deemed suitable for more detailed internal examination and were collected for necropsy to determine cause of death. Minimum estimated neonatal mortality varied widely by year and ranged from 3 to 25%. The highest number of dead pups, nearly half of the total for the study, were found in 2005; this was also the year with the highest estimate of pups born and highest proportion of pups born that were documented dying. Infection was the leading primary cause of death in most years including 2005, when 40% of the pups died from an infectious process. The 2nd leading cause of death was malnutrition; other causes included prematurity and dystocia. This study documents some of the major annual differences that can occur in both mortality rates and causes of death in Harbor Seals.

Key words: Harbor Seal, health, mortality, Phoca vitulina, pups, Smith Island, Washington

Harbor Seals (*Phoca vitulina*) are effective sentinels of ecosystem health because their longevity and extensive fat stores record the accumulation of toxins and contaminants (Wells and others 2004; Bossart 2006). This species is the most common and widely distributed pinniped in Washington's coastal waters (Jeffries and others 2003). They spend parts of their lives in coastal environments and on land, thus making them more accessible for research than many other marine species. Unlike other marine mammals, they are generally non-migratory and display strong site fidelity, remaining within the same general region throughout their life to forage and reproduce (Pitcher and McAllister 1981). Local movements within a region are influenced by weather, season, tides, food availability, and reproduction (Bigg 1981).

Monitoring the health of local Harbor Seal populations can provide information essential to conservation and management of many species in the region. Some pathogens that exist in seal populations have the potential to threaten the health of other marine mammals, and terrestrial consumers such as the Bald Eagle (*Haliaeetus leucocephalus*). In some instances, seals can serve as reservoirs of potential zoonotic pathogens, thus posing a possible health risk to humans (Gibson and others 2011; Kersh and others 2012). Seals and other marine animals are also exposed to bacterial (*Salmonella, Campylobacter, Vibrio* spp., for example) and protozoal (*Giardia, Cryptosporidium,* and *Toxoplasma* spp.) pathogens from anthropogenic sources such as agricultural and urban run-off (Miller and others 2002; Kreuder and others 2003; Stoddard and others 2005, 2008; Miller and others 2006; Fayer and others 2011).

Previous studies have examined causes of morbidity and mortality in Harbor Seals and other marine mammals of Washington State coastal waters (Calambokidis and others 1978, 1985; Steiger and others 1989; Hong and others 1996; Norman and others 2004). Past studies of Harbor Seals have compared birth rates, mortality rates, and causes of death at numerous sites in the region, including Smith Island, over a single year (Calambokidis and others 1978, 1985; Steiger and others 1989). Harbor Seal abundance in Washington increased from the 1970s through the 1990s and then stabilized at near carrying-capacity levels (Calambokidis and others 1985; Jeffries and others 2003). Harbor Seals have proved to be important indicators of polychlorinated biphenyl (PCB) and other chemical contaminant accumulation in the Pacific Northwest, and while levels of some contaminants have declined over time, other emerging contaminants (polybrominated diphenylethers, or PBDEs) have increased over the last 20 years (Calambokidis and others 1991; Ross and others 2004, 2013).

Understanding trends in mortality can help determine the relative contribution of disease, malnutrition, or other factors while establishing a baseline of mortality data for a population. Assessing causes of mortality over multiple years provides a unique opportunity to monitor these trends and identify unusual changes and contributors of mortality over a longer period of time compared to a single mortality event. This study combined active searches for dead seal pups at an important haulout site over multiple years with detailed examinations to evaluate changes in causes of mortality and monitor unusual trends, patterns, and emerging pathogens. Our specific objectives were to: (1) assess whether primary causes of mortality varied significantly between years; (2) determine if

pup size (measured by weight, length, and sternal blubber thickness) is associated with cause of mortality; and (3) evaluate whether any diseases or conditions are consistently prevalent in this study population.

#### METHODS

# Study Site

Smith Island was chosen as the study site because it is subject to relatively low levels of human disturbance. This is due to the island being 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 2010). Smith Island is a small, rocky island located within the eastern Strait of Juan de Fuca (UTM: 10 N 0511700mE 5352315mN, NAD 83) (Fig. 1). It is connected to the even smaller Minor Island by a spit that is accessible from Smith Island during low tide. For this study, both Smith and Minor Islands were surveyed and henceforth will be collectively referred to as Smith Island.

#### Survey Dates and Methods

Annual survey dates were chosen to coincide with pupping season at Smith Island (mid-June through mid-August) and to precede molting. Attempts were made to schedule multiple survey dates each year, approximately 2 wk apart. Dates were selected for times of 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 1 survey was conducted in 2008. Therefore, this study includes data collected from surveys conducted in 2004–2010 (Table 1).

The haulout site was reached by small boat. Surveyors were deposited at the eastern end of Minor Island and walked westward along the north and south sides of the island, staying below the high-tide line except to examine a carcass located above it. Once Minor Island was completed, surveyors either crossed the connecting spit on foot or were ferried by boat to do the same on Smith Island. Whenever possible, 2 teams of at least 2 individuals were formed to facilitate simultaneous searches of the north and south shores of the islands. If personnel were limited to 2 or 3 individuals, each island was circumnavigated by a single team.

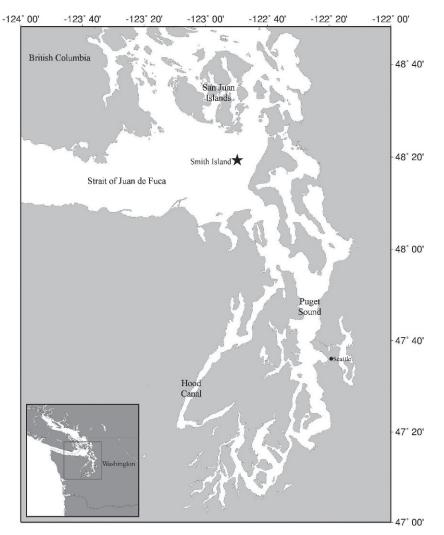


FIGURE 1. Location of Smith Island, Washington State.

	2004		2005		2006		2008		2009		2010	
		#		#		#		#		#		#
Date	Day	Dead	Day	Dead	Day	Dead	Day	Dead	Day	Dead	Day	Dead
June (16–30)	21	1(1)									29	3 (2)
July (1–15)	30 9	1(1) 4(1)	5	13 (7)	6	2 (0)			8	12 (5)		
	15	1 (1)	6	4 (3)	12	17 (8)						
			10 11 13	13 (3) 2 (2) 13 (2)								
July (15–31)			25	24 (12)					22	11 (4)	23	8 (1)
August (1–15)			7	37 (7)			5	30 (6)	20	27(1)	10	22 (6)
August (15–31) Totals	5	7 (4)	10	6 (35)	19	9 (8)	3	0 (6)		27 (1) ) (10)	33	6 (9)

TABLE 1. Survey dates and number of dead Harbor Seal pups counted and collected for necropsy (in parentheses) from Smith Island, Washington. No surveys were conducted in 2007.

All dead seals were recorded and photographed. In some cases, carcasses were inaccessible due to proximity to nesting gulls; photos of the animal were taken at a distance and the carcass was included in the count. Once counted, accessible carcasses that were not being collected were marked by biodegradable string applied at a secure point (hind flipper insertion) on the carcass to prevent duplicate counts on future surveys. Detailed locations were noted for those that were inaccessible and were compared to photos and location details of carcasses found in the same areas during subsequent surveys to avoid duplication.

Estimates of the number of Harbor Seals using Smith Island, as well as the number of pups born, were generated from counts made from aerial surveys conducted by Washington Department of Fish and Wildlife and the National Marine Mammal Laboratory. Methods for these surveys are described in Jeffries and others (2003); they involved over-flights at 700 to 800 ft (213 to 244 m) elevation and 80 knots (92 mph), and relied on counts of seals made from photographs taken. Aerial surveys and dead pup searches were not conducted on the same dates, as the presence of surveyors on the island flushes the majority of seals into the water. Estimates were not calculated for 2009 because the surveys took place long after pupping season and no live pups were sighted.

## Data and Sample Collection

Cascadia Research Collective (CRC) is a member of the Northwest Region Marine Mammal Stranding Network (National Oceanic and Atmospheric Administration [NOAA], National Marine Fisheries Service [NMFS]), and collects Level A data on all stranded marine mammals they examine, including animals found during active searches such as those in the present study. Level A data collected for all carcasses recovered during our surveys include date and time of stranding, species, age class, carcass condition, sex, weight, standard length (measured from tip of snout to tip of tail), and evidence of human interaction. In addition, blubber thickness and axillary girth were measured, when feasible. Blubber thickness was measured ventrally at the sternum. Axillary girth was measured around the animal at the axillae of the front flippers. Only relatively fresh, minimally-scavenged carcasses were collected for complete necropsy.

#### Necropsy Procedures

Whole carcasses collected for necropsy underwent complete external and internal examinations and dissections per established protocols (Pugliares and others 2007). Due to scavenging, however, it was often not possible to accurately measure certain features, such as axillary girth, so blubber thickness, weight, and length were used for comparison. Pups were classified as premature if  $\geq 50\%$  body covering was lanugo, the soft white coat covering newborn seals that is typically shed in utero in Harbor Seals (Riedman 1990). All major organ systems were examined, sampled, and photographed when lesions or other abnormalities were noted. Equal numbers of tissue samples were frozen and fixed with formalin. For histological analyses, tissues were processed using conventional methods, embedded in paraffin, sectioned to a thickness of 3-5 µm, and stained with hematoxylin and eosin. Additional samples from lesions and fluids were collected for immunohistochemistry, serology, bacterial and viral culture, antibiotic sensitivity testing, and PCR assays.

## Determining Causes 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 and others 2005). In order to determine the cause(s) of mortality for necropsied pups, initial stranding data and photographs, necropsy photos and notes, and histopathology reports were examined. Most significant in determining cause of mortality were results of the pathology report. Additional information from initial stranding response forms and necropsy notes was used as needed for clarification. The causes of mortality were divided into 4 major categories: (1) stillborn/ dystocia - stillborn pups or those that died as a result of a traumatic birth; (2) malnutritionemaciation - pups that died as a result of starvation; (3) infectious - pups that died as a result of bacterial, viral, or parasitic infection; and (4) undetermined - necropsy revealed no significant findings, or those for which

TABLE 2. Annual Harbor Seal pup counts on Smith Island, Washington, with calculated birth and minimum mortality rates.

	Highest cou		Total dead pups found,	No. of dead before max	Minimum	Minimum pup	Apparent pups	
Year (A)	Date (B)	No. of pups (C)	all surveys (D)	pup count date (E)	pups born = $C + E (F)$	mortality rate = $D \div F(G)$	not dying = F - D (H)	
2004	11-Aug	245	7	7	252	3%	245	
2005	4-Aug	352	106	69	421	25%	315	
2006	10-Aug	205	19	19	224	8%	205	
2008	15-Aug	351	30	0	351	9%	321	
2009	-	_	50	_	-	-	-	
2010	26-Jul	252	33	11	263	13%	230	

\* Live pup count represents the maximum from 1–3 aerial surveys conducted in late July to early August each year by WDFW. No annual surveys were conducted during pupping season in 2009.

post-mortem decomposition or carcass condition hindered histopathological examination.

# Mortality by Year

## Analyses

Annual minimum mortality rates were calculated using the total number of dead pups counted as a percentage of the total number of pups born that year (Calambokidis and others 1985; Lambourn and others 2010). The total number of pups born was calculated using the highest number of pups observed at the time of the survey, plus the total number of dead pups found prior to the survey. The exact chi-squared test was used to evaluate the association of year with primary causes of mortality; and mean measures of pup size, length, weight, and sternal blubber thickness (SBT) were compared using analysis of variance (ANOVA) (Stata 10.1, StataCorp, College Station, TX) and post hoc Tukey's HSD test. P-values <0.05 were considered statistically significant.

#### RESULTS

From 2004 through 2010, we found and examined 245 dead pups during 21 site surveys. Of these, 72 were suitable for necropsy (Table 1). Of the 245 dead pups, 27 were premature, 9 of which were necropsied. Length was obtained for 166 of the 245 (68%) dead pups. Mean standard length was 79.7, s = 5.1 cm (range 72 to 92 cm) for premature pups (n = 21), and 80.6, s = 6.3 cm (range 62 to 102 cm) for full-term pups (n = 143). The remaining 2 pups were weaned pups found late in the season. Sex was determined in 159 of the 245 pups collected (84 male, 75 female). Scavenging and carcass inaccessibility precluded sex determination for the remaining 86 pups.

The number of pups found dead varied by year; more than twice as many dead pups were found in 2005 than in any other year (106 in 2005 compared to 7 to 50 in any other year; Tables 1 and 2). While there were more frequent searches in 2005 (n = 7, and 5 were conducted within an 8-d window in early July) than in other years, the increased number of searches was in response to the higher number of dead seals found and the desire to collect fresher animals for necropsy. We do not believe that the higher number of pups found in 2005 is an artifact of increased effort, since most of the dead pups would have been found with fewer searches because they were found at or above the high-tide line and maximum tide levels were dropping through the period of heightened effort.

Counts of total numbers of seals using Smith Island (calculated from photos taken during 1 to 3 aerial surveys/y) during the pupping season varied annually, ranging from 370 to 2080 individuals (WDFW, unpubl. data). We calculated annual minimum mortality rates using the total number of dead pups recovered and the minimum estimate of pups born that year (derived from the maximum number of pups counted during aerial surveys, plus any dead pups found prior to the maximum count date) (Table 2). Minimum neonatal mortality ranged from 3 to 25% ( $\bar{x}$ = 11.6). The highest mortality rate (25%) occurred in 2005 and was markedly higher than in all other years (3 to 13%). While there did not appear to be a great increase in the total number of seals that year, aerial surveys did show a higher number of pups in 2005 than in other years (WDFW, unpubl. data).

### WINTER 2013

Stillborn, dystocia

Infection, metabolic

Undetermined

Total examined

Malnutrition, emaciation

1

0

1

0

0

0

4

11

14

7

8

4

0

35

0

1

5

0

1

0

6

0

5

3

3

0

0

10

1

3

4

2

3

0

9

15

25

24

72

15

8

0

24

TABLE 3. Primary (P) and contributing (C) causes of mortality by year for Harbor Seal pups recovered from Smith Island, Washington. Some pups had >1 contributing cause of mortality.

2

2

4

2

0

0

8

#### Causes of Mortality

Primary cause of mortality was determined for 48 of the 72 (67%) seals necropsied. In some cases, secondary or contributing causes were also determined (Table 3). There was a significant difference in primary causes of death among the study years (P = 0.003). In 2004, 2 of the 4 seals that were necropsied were determined to be stillborn. This was evidenced by the observation of retention of 1 pup in its fetal sac, and the lack of lung flotation in formalin for both pups, indicating that the pups had not respired. Malnutrition was the primary cause of death for 1 other pup that year. In 2005, 14 of 35 (40%) pups necropsied died as a result of infectious processes. Malnutrition was the second highest (31%) primary cause of mortality that year. In 2005, for 8 of 35 (23%) pups examined, malnutrition was a secondary cause of mortality. In 2006, 2 pups died from infectious disease as the primary cause and malnutrition as the secondary cause, and 2 others died as a result of malnutrition. In 2008, all but 1 of the pups collected were too decomposed to determine cause of mortality. That pup died from an infectious process. In 2009 and 2010, infection was again the leading cause of mortality in 5 of 10 (50%) and 3 of 9 (33%) pups respectively, while malnutrition was the leading contributing or secondary cause (50%) in 2009. Cause of mortality was determined for 6 of the 9 (67%) necropsied lanugo pups. Stillbirth/dystocia and malnutrition were the primary causes of death in 2 pups, while the remaining 4 died from an infectious process.

## Pup Size Relative to Cause of Mortality

Pup length did not vary significantly by primary cause of mortality ( $F_{3,63} = 1.22$ , P =0.310). However, pup weight did vary

significantly with cause of mortality ( $F_{3,60}$  = 5.11, P = 0.003), as did sternal blubber thickness  $(F_{3.62} = 9.30, P < 0.001)$ . These 2 factors were closely correlated (for 60 animals with both measurements, r = 0.75, P < 0.001, see Fig. 2). Among primary causes of death, mean weight was significantly lower in malnourished pups  $(\bar{x}=7.20 \text{ kg}, s=1.17)$  versus pups dead of stillborn/dystocia ( $\bar{x}$ = 10.29 kg, s = 1.63) (Tukey HSD test, P = 0.002). Mean blubber thickness of pups with malnutrition as the primary cause of death ( $\bar{x}$  = 0.57 cm, s = 0.30) was significantly thinner than those that died of: (1) stillborn/dystocia ( $\bar{x}$ = 1.4 cm, s = 0.31, P < 0.001; (2) infectious process ( $\bar{x}$ = 0.92 cm, s = 0.46, P = 0.026; and (3) undetermined ( $\bar{x}$ = 1.13 cm, s = 0.40, P = 0.001) cohorts. There was also a significant difference between the mean blubber thickness of pups in the infectious cause of death and stillborn/dystocia categories (P =0.016).

## Pathogens detected

Significant pathogens were isolated in several pups. Five pups tested positive for phocine herpes virus (PhHV-1), based on polymerase chain reaction (PCR). Three of these pups were collected in 2005, and 1 each in 2009 and 2010. In all cases, the cause of mortality was infection, with all of the pups exhibiting multiple bacterial infections. Bacteria isolated from the herpesvirus-positive pups included Actinomyces, Escherichia coli, Salmonella spp., Streptococcus bovis, Streptococcus canis, Streptococcus phocae, and alpha hemolytic Streptococcus spp. Streptococcus canis infections were detected by culture in 2 seals recovered in 2005. Salmonella typhimurium was isolated from 4 seal pups in 2005 (including a seal co-infected with S. canis), but was not detected in other years of this study.

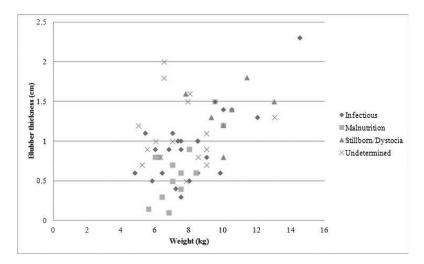


FIGURE 2. Primary cause of death in Harbor Seal pups by blubber thickness (cm) and weight (kg).

#### DISCUSSION

The higher neonatal mortality documented in 2005 cannot be explained by a disease outbreak and appears to be linked to the higher birth rate that year, which may possibly be a pattern at this site. While infectious disease was slightly more prevalent in 2005 (primary cause of death in 40% of pups examined) compared to all other years combined (primary cause of death in 33% of pups examined), we found that no single pathogen accounted for an increase in mortality. Although there did not appear to be a higher number of seals in 2005 (all age classes included) compared to other years, we estimated a minimum of 421 pups born in 2005, which was 28% higher than in the next highest year (2008). A high neonatal mortality rate has been previously documented at this site; Calambokidis and others (1978) reported a 19.7% birth rate and a pup mortality rate of 12.2% in a single year, and another single-year study conducted several years later calculated a birth rate of 26% and a 31% mortality rate (Calambokidis and others 1985; Steiger and others 1989). Annual pup mortality at Smith Island may be highly variable and influenced by a number of factors, such as overall birth rate, maternal age at pupping (Calambokidis and Gentry 1985; Steiger and others 1989), and prey availability.

The primary causes of mortality identified in this study were malnutrition and infection, which are consistent with those previously reported at Smith Island (Steiger and others 1989). While prematurity, stillbirth/dystocia, and malnutrition were prevalent in this study as well as the previous study, a higher proportion of pups appeared to have succumbed to infection during this study than previously reported at this site (Steiger and others 1989). Sampling bias may exist in this study as only fresh, minimally scavenged carcasses were collected for necropsy. Bald Eagles, the primary scavengers of Harbor Seals at Smith Island, appear to be less likely to consume emaciated pups or those with systemic infections and instead target newborn (<1-d old) and stillborn pups (Lambourn and others 2010). Consequently, a disproportionate number of individuals that died of emaciation or infectious diseases may have been left intact on the beach for collection.

Relationships between body mass and sternal blubber thickness and cause of mortality were expected. Because blubber thickness and weight are indicators of pup health, it is not surprising that pups with lower weights and inadequate blubber thicknesses were sick or nutritionally compromised, while more robust animals were either stillborn or died as a result of trauma at the time of parturition. Discerning whether emaciation was the primary cause of death rather than a contributing factor was sometimes difficult, especially in cases where both malnutrition and infection contributed to mortality. Malnutrition can lead to weakened immunity, resulting in infection (Ross and others 1993). Alternately, animals weakened by infection can become anorexic and succumb to malnutrition.

The proportion of premature pups recovered during this study was lower than previously recorded in a single year at this site. Steiger and others (1989) conducted more frequent searches prior to and early in the pupping season. Therefore, they may have been more successful in recovering early-season premature births.

Studies have demonstrated that although PhHV-1 appears to be endemic in Pacific Harbor Seal populations, fatal infections are typically associated only with neonates (Gulland and others 1997; Harder and others 1997; Goldstein and others 2003). The percentage of wild seal populations infected with PhHV-1 is currently unknown. Infected seal pups from this study were included in a recent review of all PhHV-1 cases presenting in British Columbia, Canada, and Washington State (Himworth and others 2010). Cases often presented with other simultaneous bacterial infections that could have been the primary pathogen(s), with the latent PhHV-1 infection occurring as a result of a concomitantly taxed immune system (Gulland and others 1997). This provides a challenge in distinguishing the role PhHV-1 may have played in the mortality of the seals in the present study. While PhHV-1 could have predisposed the seals to other virulent infections, these infections could have weakened pup immunity and subsequently increased the pathogenicity of PhHV-1. Regardless, PhHV-1 appeared to contribute to pup mortality at Smith Island.

Streptococcus canis and Salmonella typhimurium were unusual findings in this study and have not been previously reported at this site. It is unclear what may have contributed to the presence of S. canis in this population, because Smith Island is fairly isolated and this bacterium typically involves close contact with an infected canid (Bert and Lambert-Zechovsky 1997). In both cases, this isolate was considered important and likely represented an environmental source of infection via the umbilicus that may have originated from exposure to carrier seals, unauthorized visitors such as boaters and their dogs, or sewage runoff from the coastal mainland. A point source was not determined for S. typhimurium, which was only isolated in this population in 2005. In all 4 cases, this finding was biologically significant, as this bacterium directly contributed to mortality. The number of pups found with *S. typhimurium* is of concern, as it may represent an environmental exposure such as untreated sewage or agricultural runoff or exposure from other wildlife (Simental and Martinez-Urtaza 2008). As with most bacterial and viral pathogens, suppressed immunity caused by malnutrition or stress may have led to an increased susceptibility to infection. Because only dead animals were sampled in this study, the effect these isolated pathogens may have on live animals is unknown.

The high neonatal mortality rates we report here based on recovered carcasses, still likely represent a major underestimate and should be considered minimums. Not all dead animals would be guaranteed to wash up or to remain on the shore to be found, and it is highly probable that some would be consumed by scavengers before detection. For cetaceans especially, carcass counts can underestimate true mortality by an order of magnitude or more (Williams and others 2011). Given that Harbor Seal mortality at this site often occurs on shore, we would expect the recovery rates here to be higher than those of cetaceans; however, actual mortality would still be under-represented in the current study.

While this is one of the first multiple-year documentations of neonatal mortality rates in Harbor Seals, rates reported in some other pinniped species are either lower or similar to what we found and are not out of line with other studies on terrestrial mammals. Among phocid seals, neonatal mortality rates were estimated at 14-23% in Grey Seals (Halichoerus grypus) in the western United Kingdom (Baker 1984), and as averaging 3.8% over a 10-y period in Southern Elephant Seals (Mirounga leonina) on Marion Island, based on recovered carcasses (Pistorius and others 2001). Rates among otariids are more variable: reported neonatal mortality of South American Fur Seals (Arctocephalus australis) at Guafo Island, Chile (Seguel and others 2013), and Northern Fur Seals (Callorhinus ursinus) on the Pribilofs (Towell 2006) is well under 10%, but higher rates, more similar to what we found in Harbor Seals, have been documented in New Zealand Fur Seal (Arctocephalus forsteri) and Sea Lion (Phocarctos hookeri) populations (Mattlin 1978; Castinel et al. 2007). In terrestrial mammals, high rates of 40–50% were found in some populations of Mule Deer (*Odocoileus hemionus*; Pojar and Bowden 2004) and Reindeer (*Rangifer tarandus*; Skogland 1985). Monard and others (1997) reported varied annual rates comparable to ours, ranging from 0 to 35% over a 15-y period in wild Horses (*Equus caballus*).

This study documents relatively high rates of neonatal mortality at a Harbor Seal haulout site, but also shows how these rates and the causes of death can express significant annual variation. Understanding these patterns is critical to the detection of population shifts and disturbances.

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WINTER 2013

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# 208 NORTHWESTERN NATURALIST

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