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MORTALITY OF HARBOR SEAL PUPS AT DIFFERENT SITES IN THE INLAND WATERS OF WASHINGTON

Gretchen H. Steiger, ' John Calambokidis, ' James C. Cubbage, ' Douglas E. Skilling,² Alvin W. Smith,² and David H. Gribble³

¹ Cascadia Research Collective, Waterstreet Bldg., 218½ West Fourth Avenue, Olympia, Washington 98501, USA
² College of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97331, USA
³ Evergreen Professional Services, 19310 Northcreek Pkwy., Suite 102, Bothell, Washington 98011, USA

ABSTRACT: We examined the mortality rates and causes of death of harbor seal (*Phoca vitulina*) pups in three regions of the inland waters of Washington (USA) in 1984. One hundred eight pups were collected during 239 searches of the shoreline areas near harbor seal haulout sites or through public reports. Minimum neonatal (up to 1 mo after birth) mortality rates at these regions ranged from 12% to 26% of the pups born. Neonatal mortality was highest in the Strait of Juan de Fuca; 33 of the estimated 105 (31%) pups born at the primary site died. Causes of death varied by location. In southern Puget Sound predation by coyotes (*Canis latrans*) was the primary cause of death, accounting for eight of 43 (19%) of the dead pups examined; starvation was the next most common cause of death. Mortality at study sites in the Strait of Juan de Fuca was related to premature parturition; 19 of 49 (39%) of the pups found dead were born prematurely. Nine species of bacteria were identified in samples taken from 42 pups; *Proteus* sp. and *Escherichia coli* were the most common.

Key words: Neonatal mortality, harbor seal, *Phoca vitulina*, mortality rates, causes of death, microbiology, predation, starvation, field study.

INTRODUCTION

Systematic studies on neonatal mortality of pinnipeds have focused on species that have stable rookeries where pups remain on land during the first months of life; these included Antarctic fur seals (Arctocephalus gazella) (Baker and Doidge, 1984; Doidge et al., 1984), northern fur seals (Callorhinus ursinus) (Keyes, 1965), New Zealand fur seals (Arctocephalus forsteri)(Mattlin, 1978), northern elephant seals (Mirounga angustirotris) (Le Boeuf and Briggs, 1977; Huber et al., 1984), southern elephant seals (Mirounga leonina) (Carrick et al., 1962), and grey seals (Halichperus grypus) (Summers et al., 1975; Anderson et al., 1979). Studies on neonatal mortality of harbor seals have been more limited. Causes of death of stranded harbor seals, including some pups, have been reported by Stroud and Roffe (1979) and Johnson and Jeffries (1977, 1983). Rates of harbor seal neonatal mortality determined in field studies have been reported at Sable Island, Nova Scotia, Canada (Boulva, 1971; Boulva and McLaren, 1979) and at one site in central California (USA) (Allen, 1980). Estimates of juvenile mortality of harbor seals used in population models or life tables have varied greatly, between 20% and 65% (van Bemmel, 1956; Bigg, 1969; Reijnders, 1978; Reijnders et al., 1981b). This variation reflects the uncertainty and lack of field data available for this species.

This study compares rates and causes of neonatal mortality of harbor seals, determined using systematic searches of haulout sites, among three regions in the inland waters of Washington (USA).

MATERIALS AND METHODS

Beach searches for dead seals were conducted in three regions: southern Puget Sound (Puget Sound south of Seattle), the Hood Canal, and the Strait of Juan de Fuca (Fig. 1). Our efforts were concentrated at one primary haulout site within each region: Gertrude Island (47°13'N, 122°39'W) in southern Puget Sound, Skokomish Delta (47°21'N, 123°07'W) on the Hood Canal, and Smith Island (48°19'N, 122°50'W) in the Strait of Juan de Fuca. Effort by site is reported



FIGURE 1. Study sites for determining harbor seal pup mortality in the inland waters of Washington.

in Table 1. Beach searches extended from several months prior to the onset of pupping until mother and pup pairs were no longer observed. They were conducted between: 6 March and 21 November 1984 in southern Puget Sound, 15 January and 7 November at the Hood Canal, and 4 May and 29 August in the Strait of Juan de Fuca, corresponding to the varied timing of harbor seal parturition in these areas (Calambokidis et al., 1985). Searches were conducted at times when harbor seals were not hauled out. One or more persons walked the haulout and

	Searc	ches		Neonatal	Post-			
Site	Number	Hr	Pups born	Number	%	mortality		
Southern Puget Sound			-					
Gertrude Island	70	76	83	14	17	2		
Henderson Inlet	13	24	60	8	13	6		
Eld Inlet	6	13	13	2	15	_		
Budd Inlet	10	5	8	2	25	_		
Total SPS	99	118	164	26	16	8		
Hood Canal								
Skokomish Delta	27	65	63	9	14			
Duckabush Delta	22	20	37	4	11			
Dosewallips Delta	18	29	29	2	6.9	_		
Total HC	67	114	129	15	12	_		
Strait of Juan de Fuca								
Smith Island	45	63	105	33	31	_		
Protection Island	28	13	63	11	17	_		
Total Strait	73	76	168	44	26	_		
All locations monitored	239	308	461	85	18	8		
Other sites*	0	0	_	8		7		
Total pups found				93		15		

TABLE 1. Search effort, neonatal (pre-weaning, up to 1 mo after birth) and postweaning (up to 3 mo after birth) mortality by site. Number of pups born used to determine neonatal mortality rates was reported by Calambokidis et al. (1985).

• Other sites where searches were not conducted but were within study regions (animals reported through the Stranding Network).

surrounding areas; nearby areas were searched by skiff. Additionally, we were notified of marine mammal strandings as a participant in the Northwest Stranding Network (National Marine Fisheries Service, Seattle, Washington 98115, USA) that coordinates all stranding reports from the public.

Between 4 May 1984 and 8 January 1985, 108 dead harbor seal pups were found. Fifteen of these animals were determined to be weaned by tooth development and date collected relative to the timing of pupping. External and internal examinations were conducted by trained personnel; standard measurements were taken as described by Scheffer (1966). The extent to which we could thoroughly examine and sample an animal depended upon its postmortem condition. Portions of salivary glands, thyroid, heart, lung, liver, pancreas, spleen, adrenal glands, kidney, stomach, colon, gonads, skin, skeletal muscle, blubber, thymus, and umbilicus were collected and fixed in 10% buffered formalin. Tissues from 47 pups were fixed using standard histological methods and examined using light microscopy (Evergreen Professional Services, Bothell, Washington 98011, USA).

Dacron swabs (American Scientific Products,

McGaw Park, Illinois 60085, USA) of the nose, throat, and anus of 49 freshly dead or moribund pups were collected and placed into 2-dram vials of tissue culture media (MEM; Whittaker Bioproducts, Inc., Walkersville, Maryland 21793, USA) for virology. At Oregon State University (College of Veterinary Medicine, Corvallis, Oregon 97331, USA), samples were processed immediately upon receipt or were frozen at -85C until cultured. Swabs in vials were vortexed and then clarified by centrifugation at 2,000 g for 10 min. A 0.2-ml subsample of the resulting supernatant was placed in each of three roller tubes containing monolayers of Vero Monkey Kidney, Porcine Kidney, or Crandall Feline Kidney Cells (American Type Culture Collection, Rockville, Maryland 20852, USA). After 1 hr absorption of the "dry" monolayer, tubes were fed with 1.5 ml of MEM containing 2% fetal bovine serum and antibiotics; penicillin (GIBCO, Cleveland, Ohio 44194, USA), streptomycin (GIBCO), and gentamicin (Sigma Chemical Company, St. Louis, Missouri 63178, USA). Tubes were incubated at 37 C on a roller drum and were observed daily by light microscopy for cytopathic effect (CPE). Cultures demonstrating 3+ to 4+ CPE (75 to 100% of the

cell monolayer visibly infected by the virus) were freeze-thawed, vortexed, clarified and repassaged. Cultures not showing CPE after 10 days were frozen; then thawed, vortexed, clarified by low-speed centrifugation, and repassaged. Each culture was passaged at least three times on each cell line. Twenty-four randomly selected virology specimens (original swab samples) were processed for negative stain electron microscopy (Skilling et al., 1985).

Samples were collected for general bacterial culture and specifically for Leptospira interrogans. Forty-two pups were sampled for bacterial examination. Culturette (American Scientific Products, McGaw Park, Illinois 60085, USA) swabs of brain, liver, kidney, eye, or respiratory fluid were cultured for isolates at Oregon State University (Veterinary Diagnostic Laboratory, Corvallis, Oregon 97331, USA). Samples for isolating the leptospira spirochete were taken after we found a large number of premature pups on Smith Island. Liver and kidney were collected into leptospiral medium (provided by A. B. Thiermann, National Program Leader Animal Health, Beltsville, Maryland 20705, USA) from 23 freshly dead pups and examined at the College of Veterinary Medicine (Oregon State University). Tissue samples for possible leptospiral isolation were processed using methods described in Smith et al. (1974a). Tissue was ground in a sterile Ten Broeck grinder (VWR Scientific, San Francisco, California 94120, USA) diluted with phosphate buffered saline, and inoculated into tubes containing leptospiral growth (semisolid) medium. Cultures were incubated at 30 C and were examined by darkfield microscopy. At the time of preparation, a drop of each ground tissue was also observed by darkfield microscopy for the presence of leptospires.

Minimum mortality rates were calculated using the number of dead pups found as a percentage of the pups born. Calambokidis et al. (1985) reported the number of pups born at all our study sites as part of a broader study that examined harbor seal population dynamics, which included over 1,400 hr of harbor seal censuses. The total number of pups born was calculated using the highest number of pups seen at one time, plus the number of dead pups found before the highest count, plus the number of births that occurred after the highest count. Further details are reported in Calambokidis et al. (1985). Chi-square and correlation analyses were conducted as described in Zar (1984).

Information from macroscopic and microscopic examinations was used to determine primary and contributing causes of mortality. Stillbirth and premature births were classified as primary causes of death; the ultimate causes for these conditions were not known. For four pups that were premature and stillborn, we arbitrarily designated premature birth as the primary cause of mortality and stillbirth as the contributing cause.

RESULTS

Mortality rates

Observed harbor seal neonatal (first month after birth) mortality was significantly different among regions ($\chi^2 = 7.2$, P < 0.05). The rate varied from 16% in southern Puget Sound, 12% in the Hood Canal, and 26% in the Strait of Juan de Fuca (Table 1); there was no significant difference in the rate of pup mortality among sites within each region ($\chi^2 = 0.37$, P > 0.05 in southern Puget Sound; $\chi^2 =$ 0.88, P > 0.05 in the Hood Canal, $\chi^2 =$ 2.1, P > 0.05 in the Strait of Juan de Fuca). The highest neonatal mortality rate was at Smith Island, where a minimum of 31% of the pups died. Differences in search effort were not responsible for the different mortality rates observed among sites; there was no correlation between observed mortality rates and either number of searches (n = 9, r = 0.279, P > 0.05) or hours searched (n = 9, r = 0.180, P > 0.05). In addition to neonates, 15 weaned pups were found up to the third month after birth and are reported in Table 1. Only one was found at the haulout area.

Mortality rates determined are minimums because some carcasses possibly washed away or were eaten by scavengers. Tides and currents would most likely affect the number of dead pups at the two island sites in the Strait of Juan de Fuca, where carcasses that washed away probably would not be recovered. Because these exposed sites had the highest mortality rates, the regional differences we found are not an artifact of these circumstances. Scavenging on pup carcasses occurred most often on the Hood Canal. Of the neonates found from the primary study sites, seven of nine (78%) showed evidence of scavenging at Skokomish Delta (Hood Canal), nine of 14 (64%) at Gertrude Island (southern Puget Sound), and 11 of 33 (33%) at Smith Island (Strait of Juan de Fuca). Dead

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	Souther Sou	n Puget ind	Hood Canal		Stra Juan d	it of e Fuca	All sites		
Cause of death	Р	С	Р	С	Р	С	Р	С	
Premature	2	1	1	0	19	0	22	1	
Stillborn	3	1	3	0	4	3	10	4	
Emaciation									
Pre-weaning	3	0	2	0	9	1	14	1	
Postweaning	3	0	0	0	0	0	3	0	
Coyote kill	8	0	0	0	0	0	8	0	
Incidental catch-fisheries	5	0	0	0	0	0	5	0	
Shot	1	0	0	0	0	0	1	0	
Other trauma	3	0	1	0	2	0	6	0	
Drowning ^b	2	5	0	0	0	1	2	6	
Septicemia	2	3	0	0	2	3	4	6	
Other suspected infectious agents	0	4	0	0	1	2	1	6	
Myocardial necrosis	0	0	0	0	1	0	1	0	
Pneumonia	0	1	0	0	0	4	0	5	
Euthanasia or died in captivity	2	0	0	0	1	0	3	0	
Not determined or unsuitable	9	—	9	—	10		28		
Total examined	43		16		49		108		

TABLE 2. Primary (P) and contributing (C) causes of death of harbor seal pups (neonatal and weaned) by region in 1984.

* Premature stillborn pups are listed as premature for primary cause of death.

^b Drowning as a primary cause does not include animals that drowned as a result of other injuries included in another category (e.g., incidental catch).

^c Lesions seen included at least one of the following: interstitial pneumonia, lymphoid hyperplasia or hypertrophy, or thymic and hypatic atrophy in a stillborn animal.

pups were scavenged primarily by turkey vultures (*Cathartes aura*) at sites on the Hood Canal, coyotes (*Canis latrans*) at Gertrude Island, and bald eagles (*Haliaeetus leucocephalus*) at Smith Island. The observed low pup mortality in Hood Canal may be an artifact of the high scavenging rate in this region because scavenged pups were less likely to be found.

The mean standard length for premature pups was 69 cm (n = 15, SD = 7.2), 84 cm for full-term pups (n = 61, SD = 5.0), and 91 cm for postweaning animals (n = 14, SD = 5.7). More male (45) than female (31) pups (premature and full-term) were collected, although this difference did not deviate significantly from parity ($\chi^2 =$ 2.6, P > 0.05).

Causes of mortality

Causes of mortality of harbor seal pups varied by region (Table 2). Premature births, characterized by early timing of birth and presence of lanugo coat, which

is normally shed in utero (Scheffer and Slipp, 1944; Stutz, 1966), were associated with the largest number of deaths (21% of the dead pups). However, 19 of 23 of these pups were from the two sites in the Strait of Juan de Fuca. Eleven percent of the pups born and 39% of those found dead were premature at these two sites. The occurrence of premature whelping in this region was significantly higher ($\chi^2 = 19.9$, P < .0001) than sites in southern Puget Sound (2% of pups born) and Hood Canal (1% of pups born). Four premature pups were stillborn, three at Smith Island and one at Gertrude Island. No viable pups with lanugo coats were observed during this study.

Starvation (emaciation, characterized by blubber thickness ≤ 0.5 cm) and stillbirths were the next two most common primary causes of mortality. The occurrence was similar among regions, although emaciation was more common at sites in the Strait of Juan de Fuca. Fifteen of 18 emaciated pups were found at haulout sites during the pupping season and appeared to be 1to 2-wk-old; these pups probably received little nourishment from their mothers.

A large number of live, newborn pups (up to 14 at one time) were observed hauled out without mothers, particularly at Smith Island. Generally, they were weak and would not try to enter the water when approached. These lethargic pups included both newborn (with fresh umbilical cords and normal blubber thickness) and emaciated animals. Three had pale yellow conjectival exudate, one was blind (*Corynebacterium* sp. was isolated from the eye). Most of these animals were later collected dead and emaciated.

Predation by coyotes was responsible for neonatal mortality at Gertrude Island. Eight pups had extensive puncture wounds and hemorrhaging in the head and neck regions and attacks on live pups were witnessed on two occasions. Tissues of four of these carcasses were examined histologically; three showed lesions of septicemia or other infectious agents and none were emaciated. This was the only site where coyote predation was observed. A detailed description of this predation will be published separately.

The causes of death of weaned animals were different from those of neonatal pups. Human-caused mortality was found in nine of 15 of these animals: five by drowning in gillnets and four by trauma attributed to a blow to the head or gunshot wound. Three weaned animals were emaciated, though they probably received nourishment from their mothers and starved after weaning.

In addition to the lesions directly associated with the causes of mortality, several incidental pathologic findings were noted. Ten of 12 pups with hepatic atrophy or unusual liver pigmentation were from Smith Island. Nine of 23 pups with thymic atrophy showed no other signs of stress; no relationship was obvious with regional variation or diagnosed causes of death. Other microscopic lesions seen in pups included: hepatitis, umbilical inflammation, cellulitus, lymphoid necrosis, thymic necrosis and reduced colloid in the thyroid.

Microbiology

The bacteria isolated from harbor seal pups are summarized in Table 3 by region. *Proteus* sp. or *Escherichia coli* were isolated from brains of 10 of 17 pups from the Hood Canal and the Strait of Juan de Fuca. Because only three of these 10 animals had signs of septicemia or other infectious agents, we suspect the prevalence of these two bacteria may be the result of contamination after death or during sampling.

Leptospira interrogans and San Miguel Sea Lion Virus (SMSV) were not found in any samples. However, virions with a morphology typical of influenza virus (genus Influenzavirus) were seen in four of 24 randomly selected pups; one in Eld Inlet, and three from Smith Island. Reovirus-like particles were seen in one sample from Smith Island in a pup that was emaciated although its stomach contained fresh milk. These particles were 80 nm in diameter and appeared identical to Reovirus isolates from California sea lions (Zalophus californianus), Steller sea lions (Eumetopias jubatus), and northern fur seals in the northern Pacific (A. W. Smith, D. E. Skilling and S. Poet, unpubl. data).

Discussion

The neonatal mortality rates we found were higher than have been reported previously for harbor seals but are within the range reported for other pinniped species. Natural pre-weaning mortality for harbor seals at Sable Island, Nova Scotia was 12% (Boulva, 1971; Boulva and McLaren, 1979). Allen (1980) reported a rate of 7% mortality at Double Point, California. Reported neonatal mortality rates for other pinniped species (otariids, elephant seals, and grey seals) vary widely and were between 2 and 61% (Carrick et al., 1962; Keyes,

	Sout	Southern Puget Sound		Hood Canal		Strait of Juan de Fuca				All regions					
Description	В	L	R	В	L	В	L	R	K	E	В	L	R	K	E
Number sampled	17	14	6	3	3	14	7	2	2	1	34	24	8	2	1
No. significant isolates	7	7				3	1		—	—	10	8		_	_
Escherichia coli	1	2	1		_	7	5	1	2	—	8	7	2	2	
Corynebacterium sp.				—		_	_	—	—	1	_	_			1
Pasteurella hemolytica	_		1		_		_			_	-		1		
a-Streptococcus sp.	1		1			—	_	-	—	—	1		1		
β-Streptococcus sp.	2	1	—	_	_	1		—		—	3	1			—
Acinetobacter sp.	1				_		—	—	—		1	_		—	—
Proteus sp.		1	1	2-	1	8	2	1	—	—	10	4	2	—	—
Pseudomonas sp.	_	1				—	—	—	—		—	1		—	—
Enterobacter sp.	_	1	—	_	_			—		—	_	1	—		—
Mixed coliforms only	5	2	2	1	2	2	1	1	-	_	8	5	3	—	_

TABLE 3. Bacteria isolated from brain (B), liver (L), respiratory tract (R), kidney (K), and eye (E) of harbor seal pups by region. The number of tissues from which bacteria were identified are listed.

* Proteus mirabilus was isolated from one sample.

1965; Le Boeuf et al., 1972; Summers et al., 1975; Mattlin, 1978; Anderson et al., 1979; Doidge et al., 1984).

Density-dependent juvenile mortality has been demonstrated in pinniped populations (Summers et al., 1975; Doidge et al., 1984; Swartzman, 1984; York, 1985), especially at populations close to carrying capacity (Fowler, 1981). It is not known how close harbor seal populations in our study areas are to carrying capacity; however, they have been increasing at 5 to 20% a year since the late 1970's (Calambokidis et al., 1985). Information in subsequent years will provide greater insight into the role of these factors.

At Smith Island, the high mortality rate may be related to a disproportionately high number of young primiparous females. Calambokidis et al. (1985), using length measurements calculated through aerial photogrammetry and age and length data reported by Bigg (1969), found a large proportion of 2- to 4-yr-old seals at Smith Island during the whelping season in 1984 in contrast to other sites. Many of these seals were pregnant or with pups, and seemed to represent a cohort of animals reaching age of first reproduction. At Smith Island, where pup mortality was significantly higher than in other regions, pups were smaller (J. Calambokidis and G. H. Steiger, unpubl. data). For northern fur seals, pups born to younger females had higher mortality rates and were smaller than pups born to older females (Calambokidis and Gentry, 1985).

The primary causes of neonatal mortality we found were similar to those reported previously for pinnipeds, but with varying prevalence. Common causes of death included starvation, stillbirth, septicemia and pneumonia (Carrick et al., 1962; Keves, 1965; Bonner, 1970; Mattlin, 1978; Anderson et al., 1979; Boulva and McLaren, 1979; Stroud and Roffe, 1979; Baker et al., 1980; Baker and Doidge, 1984; Dierauf et al., 1986). Emaciation, the major cause of death in most studies, was less prevalent here; premature births, stillbirths, septicemia and pneumonia were seen more often in this study. Predation by coyotes on pinniped neonates has not been reported at other sites; this predation occurred more often than has been reported for other predators (Boulva and McLaren, 1979; Gentry and Johnson, 1981; Baker and Doidge, 1984).

Other mortality studies provide some insight into the ultimate causes of mortal-

ity such as starvation. Starvation in pinniped neonates has been considered the result of mother and pup separation or abandonment (Le Boeuf et al., 1972; Bonner, 1975; Boulva and McLaren, 1979). Our observations indicate that some pups were moribund prior to starvation. Similarly, Calambokidis and Gentry (1985) found that mother and pup separation was not often the cause of starvation in northern fur seal pups observed from birth until death.

The causes of the high rate of premature births in the Strait of Juan de Fuca may be related to disease agents, but probably do not involve contaminants. A high rate of premature births and birth defects were reported at Gertrude Island, in southern Puget Sound in 1970–1972 (Newby, 1971, 1973; Arndt, 1973) with pollutants suggested as the cause (Arndt, 1973). High rates of premature parturition in California sea lions have been linked to leptospirosis (Smith et al., 1974a), SMSV (Smith et al., 1974b), the organochlorines DDT and PCB (DeLong et al., 1973), or a more complex interaction among disease agents and pollutants (Gilmartin et al., 1976). The highest prevalence of premature births we found was in the region of lowest contaminant concentrations (Calambokidis et al., 1984). We cannot dismiss the role of SMSV or leptospirosis because most of the premature births occurred before we began sampling for them. Huber et al. (1984) reported a high rate of premature whelping in Steller sea lions on the Farallon Islands, California, with an influenza-like virus seen in five of seven animals. Influenza virus has been implicated as the cause of a mass mortality of over 400 harbor seals during 1979 and 1980 in New England (Geraci et al., 1982). Influenza epizootics may have been responsible for numerous historical mass mortalities of pinnipeds (Geraci et al., 1982).

The signs of septicemia or other infectious agents in pups killed by coyotes at Gertrude Island suggest they were debilitated prior to attack. Seals fled from coyotes by entering the water and moribund pups probably were slower to try to escape. Similarly, Stroud and Roffe (1979) reported several cases of shark predation on marine mammals afflicted with chronic debilitating diseases. Predation on moribund animals obviously has less impact on seal populations than if the attacks were on healthy animals.

Many of the bacteria we identified from harbor seals have been found in other pinnipeds; these include Escherichia coli, alpha-Streptococcus sp., beta-Streptococcus sp., Acinetobacter sp., Proteus sp., and Pseudomonas sp. (Bonner, 1970; Anderson et al., 1979; Baker et al., 1980; Medway, 1980; Reijnders et al., 1981a; Geraci et al., 1982; Baker and Doidge, 1984). The role of specific bacteria in any of the causes of death found is difficult to determine. Geraci et al. (1982) concluded that the inconsistent pattern in bacteria isolated from seals dying from influenza indicated that they were not a contributing cause of death. Samples from southern Puget Sound had a greater diversity of bacteria found than animals from Hood Canal or the Strait of Juan de Fuca; five species of bacteria found in samples from southern Puget Sound were not seen in other areas. Corynebacterium sp. was isolated from the infected eye of a live pup at Smith Island; Bonner (1970) also reported isolating a Corynebacterium sp. from the infected eye of grev seal.

We found variations among regions in neonatal mortality rates, causes of death, and the bacteria that were present. The three regions are in relatively close proximity; the sites are less than 150 km apart. These results indicate the regional variability in some basic biological parameters of harbor seals. Caution should be used in extrapolating results from studies on harbor seals at a single site to large geographical areas.

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