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Aerobic bacteria of northern fur seal (Callorhinus ursinus) pups on the Pribilof (USA) Islands and Medny (USSR) Island

Matthew Anderson Burd
San Jose State University

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the Pribilof (USA) Islands and Medny (USSR) Island**

Burd, Matthew Anderson, M.S.

San Jose State University, 1992

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**300 N. Zeeb Rd.
Ann Arbor, MI 48106**

**AEROBIC BACTERIA OF NORTHERN FUR SEAL (CALLORHINUS URSINUS)
PUPS ON THE PRIBILOF (USA) ISLANDS AND MEDNY (USSR) ISLAND**

**A Thesis
Presented to
The Faculty of Moss Landing Marine Laboratories
San Jose State University**

**In Partial Fulfillment of the
Requirements for the Degree
Masters of Science in Marine Science**

**by
Matthew Anderson Burd
May 1992**

APPROVED FOR THE DEPARTMENT OF
MOSS LANDING MARINE LABORATORIES

Dr. James T. Harvey

Dr. Michael S. Foster

Dr. Kurt P. Snipes

APPROVED FOR THE UNIVERSITY

ABSTRACT

AEROBIC BACTERIA OF NORTHERN FUR SEAL (CALLORHINUS URSINUS) PUPS ON THE PRIBILOF ISLANDS AND MEDNY ISLAND (USSR)

by Matthew A. Burd

Differential prevalence (%) of potentially pathogenic bacteria may contribute to differential pup mortality (%) for rookeries of the Pribilof Islands and Medny Island. Aerobic bacteria were cultured from ocular, pharyngeal, and rectal swabs of pups. Weight and index of condition of pups with Pseudomonas aeruginosa, Acinetobacter lwoffii, group-D Salmonella, and Staphylococcus aureus were less than pups without these bacteria. Pups on Little Zapadni, Zapadni, Reef, Vostochni, and Morjovi rookeries had a significantly greater number of bacterial species, and pups on Reef and East Cliffs rookeries had a significantly greater number of species of pathogenic bacteria. Linear regressions between pup mortality, number of live pups, and number of bacterial species and number of species of pathogenic bacteria were not significant, indicating most bacteria did not affect pups. Prevalence (%) of group-D Salmonella was correlated positively with pup mortality (%) and may affect numbers of pups on Little Zapadni, Zapadni, Zapadni Reef, Vostochni, and Gorbach rookeries.

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INTRODUCTION

The northern fur seal (Callorhinus ursinus) is a member of the family Otariidae and sexual dimorphism allows adults to be distinguished. Males reach 2.13 m in length and weigh about 182-272 kg, and females reach 1.42 m in length and weigh about 43-50 kg (Lander 1979). At birth, pups weigh about 4.5-5.4 kg and measure about 64 cm in length (Lander 1979). Adult males come ashore in mid-May and establish territories on rookeries, whereas younger males arrive later in the summer and are confined to hauling grounds away from the breeding areas (Peterson 1965). In June, pregnant females come ashore and within two days give birth to a single pup (Peterson 1965). From 1 to 100 ($\bar{x}$ = 60) females occur in a single male's territory (Peterson 1968). Females have a bicornuate, double-horned uterus which allows fertilization within a week after birth of her pup. Blastocyst implantation in the uterus is delayed for about four months, presumably ensuring adequate health of the female and synchronous pupping the next breeding season (Craig 1964). Pups are dependent on their mothers for food for the first three to four months of life (Gentry and Holt 1986). Five to six days after pups are born, mothers go to sea and feed returning five to six days later to suckle their pups for one to two days. This foraging-suckling cycle occurs an average 12 to 14 times during each breeding season (Gentry and Holt 1986). Pups enter the water in October and November and remain pelagic for one to three years.

Northern fur seals spend much of their lives at sea, but return to breeding grounds once a year to breed. There are six breeding populations of northern fur seals: 1) Pribilof Islands, 2) Commander Islands, 3) Robben Islands, 4) Kuril Islands, 5) Bogoslof Island, and 6) San Miguel Island (Loughlin 1989). St. Paul Island is located approximately 324 km north of Unalaska in the eastern Bering Sea. St. George Island is located approximately 64 km southeast of St. Paul Island. The Pribilof Islands (St. Paul and St. George) population is the greatest in number, consisting of about 800,000 individuals (Fowler 1985). The

Commander Islands (Bering and Medny) are located approximately 350 km west of the Kumchatka Peninsula in the western Bering Sea, and consists of about 265,000 individuals (Lander 1980)

In the early 1800's, an estimated 6 million northern fur seals occurred on the Pribilof Islands (Scheffer et al. 1984). By 1909, under heavy commercial exploitation, the herd numbered 300,000 (Lander 1980). Pup counts have been made on the Pribilof Islands since 1912 (Scheffer et al. 1984), but few data exist from 1924 to 1950. Since 1961, pup production on the Pribilof Islands has been assessed by the "shearing sample" method described by Chapman and Johnson (1968) and modified by York and Kozloff (1987). From 1950 to 1980, numbers of pups born on St. Paul Island has decreased. Current levels (1.4 million) probably are about one third what they were in the 1940's and early 1950's (Fowler 1985). From 1975 to 1983, numbers of pups born on St. George Island decreased 5.95% per year, whereas numbers born on St. Paul Island decreased 6.7% per year (York 1990). In 1988, an estimated 202,312 pups were born on St. Paul Island, significantly greater than 1987 (York and Antonelis 1988). Despite this increase in natality, the population of northern fur seals on the Pribilof Islands has remained relatively stable since 1984 (York 1990).

All causes of the fur seal decline (1950-1980) have not been identified, but may include the combined effects of harvesting females during 1956-68, entanglement in net material, weather, reduced prey availability, and disease (Loughlin 1989). The greatest influence on the population of fur seals during the past 30 years is postulated to be harvesting of females (York 1987). Fowler (1987) speculated entanglement in net material discarded from fishing vessels also contributed to the decline. Incidental catch by commercial fisheries is thought insignificant (Loughlin et al. 1983). Kenyon et al. (1954) and Lander (1979) reported starvation during prolonged periods of stormy weather as a major cause of death in

young northern fur seals. Loughlin et al. (1986) described a decline in duration of feeding trips of northern fur seals during the past 20 years, indicating changes in prey availability may have occurred.

Disease affects pinnipeds and may have contributed to the decline (1950-1980) in numbers of northern fur seals on the Pribilof Islands. An epizootic killed more than 17,000 harbor seals (Phoca vitulina) in Scandinavian, German, and Dutch waters, and a seal morbillivirus was isolated from diseased seals (Cosby et al. 1988). Rand (1975) reported thousands of Galapagos sea lions (Arctocephalus townsendii) died during a 1970-71 epizootic, and suggested Pseudomonas aeruginosa was responsible for a nodular suppurative cellulitis. Stroud and Roffe (1979) reported bacterial infection was the primary cause of death in 27% of 68 marine mammals stranded on Oregon beaches. Bacterial infection may be a more important cause of morbidity and mortality than indicated because many animals were too decomposed to obtain bacteriological samples, and animals weakened by chronic infection may be more susceptible to predation (Stroud and Roffe 1979). Viral, bacterial, fungal, and metazoan parasites have been reported in marine mammals (Keyes 1965, Wilson and Sweeney 1970, Stroud and Dailey 1978, Waldorf and Vedros 1978), and these parasites may contribute to mortality of pups of northern fur seals.

Mortality of pups may be affected by predation, trauma from adults, inclement weather, starvation, parasites, and infectious disease. Gentry and Johnson (1981) observed northern sea lions (Eumetopias jubatus) eating pups in the water near St. George Island. Emaciation syndrome and hookworm disease caused mortality of pups on the Pribilof Islands for many years (Laukner 1985). Keyes (1965) necropsied 226 pups and reported malnutrition as the primary cause of death in 37.6% of the animals and traumatic lesions in 36.7% of the animals. Keyes et al. (1975) necropsied 244 pups from three study areas on St. Paul Island. Trauma from adults, including skull fracture, liver rupture, and bite wounds

caused 2.9 % of deaths, emaciation syndrome and hookworm disease 65.1 % of deaths, and microbial infection 12.7 % of deaths. Of 90 pups necropsied, Spraker (unpl. data 1987) reported starvation and trauma (65%) were the most common diagnoses followed by infectious disease (14.5%). Smith et al. (1977) reported Leptospira pomona was associated with a multiple hemorrhage syndrome in pups. From 1964 to 1978, infectious disease was the primary diagnosis (12%) for cause of death in pups on the Pribilof Islands (Lander 1980) and mortality of pups caused by infectious disease may affect regulation of numbers of northern fur seals.

Survival of pups may contribute twofold to the regulation of northern fur seals considering increases in both pup mortality and the variance of pup mortality with population size (Fowler 1984). Microparasites (bacteria and viruses) may play a role analogous to predators; when host populations increase, direct transmission of microparasites is enhanced (Lack 1954). During periods of nutritional stress, pathogenicity of parasites is amplified contributing to density-dependent regulation of natural populations (Anderson and May 1979). Mortality of pups of northern fur seals on land is affected by disease, and probably is density-dependent (Lander and Kajimura 1976). Striking differences (3-39%) in pup mortality are possible for rookeries of the Pribilof Islands (Lander and Kajimura 1976). In 1987, the Zapadni rookery of St. Paul Island, had the greatest estimated pup mortality (6.5 %) and the Ardiguen rookery the least (Briggs 1990). In 1985, the Staraya Artil rookery of St. George Island had the greatest pup mortality (3.16 %) and the East rookery the least (1.5 %; Briggs 1990). Pinnipeds form dense aggregations on land at various times throughout the year. Harwood and Greenfell (1990) suggested increased disease transmission rates result from this behavior and predispose pinniped populations to epizootics.

Northern fur seal pups aggregate in large pods during the breeding season (Peterson 1965). This behavior is thought to reduce trauma to pups from adults, but increases the transmission of ectoparasites and other potential disease agents (Keyes 1965). Pups are born on geographically distinct rookeries and pups remain on rookeries where they were born until they leave in mid-October. Contact among pups on different rookeries is thought minimal. Therefore, pups on different rookeries may have different different pathogenic bacteria. The purpose of this study was to determine if the differential prevalence (%) of potentially pathogenic bacteria contribute to the differential pup mortality (%) for rookeries of the Pribilof Islands and Medny Island. I define a pathogenic bacterium as any bacterium that was reported capable of causing disease in Otariids and is the only definition used throughout this paper.

This study determined the species and prevalence (%) of bacteria of pups, and tested the hypotheses: 1) there are differences among rookeries in numbers of pups with potentially pathogenic species of bacteria; 2) rookeries and islands with greater relative pup mortality (%) have a greater number of bacterial species and species of potentially pathogenic bacteria per pup; 3) rookeries and islands with a greater number of live pups have a greater number of bacterial species and species of potentially pathogenic bacteria per pup; 4) pup weight and an index of condition is correlated negatively with number of bacterial species and species of potentially pathogenic bacteria per pup; and 5) pup weight and index of condition is less in pups with a potentially pathogenic bacterium than pups without a potentially pathogenic species of bacterium.

MATERIALS AND METHODS

A. Assessment of Pup Condition

Between 14-31 August 1990, three of six rookeries of St. George Island (USA), 12 of 14 rookeries of St. Paul Island (USA), and a single rookery of Medny Island (USSR) were sampled (Fig. 1). Thirty pups per rookery were selected randomly and sex, tag numbers, and morphometric measurements were recorded. Pups were placed in a bucket and weighed to the nearest 0.25 kg with a hanging scale. Standard length was measured to the nearest centimeter by placing the pup ventrally on a board and measuring a straight line from tip of snout to end of tail. Three additional measurements (measured to the nearest 0.1 cm) were determined for pups of the Pribilof Islands only: 1) cranial width, determined using a caliper to measure the distance between insertion points of each ear pinnae, 2) index of fat thickness, determined using a caliper to measure the thickness of a pinch behind the right fore-flipper, and 3) fore-flipper length, determined by bending the fore-flipper at the wrist and measuring from base of bend at the wrist to end of longest digit. An index of condition was calculated by dividing pup weight by standard length, and multiplying by the index of fat thickness.

B. Collection of Culture Swabs

Pups were restrained after measurements were determined and rectal, ocular, and pharyngeal swabs were used to collect bacteria. Ocular swabs were collected by spreading the upper and lower eyelids, and gently pressing the medial canthus allowing the nictitating membrane to unfold. A sterile rayon tipped swab (Cultureswab Transport System, DIFCO, Surrey, UK) was rolled across the palpebral conjunctiva and placed into AMIES medium without charcoal. Pharyngeal swabs were collected by opening the mouth and waiting for the pup to inhale. When a breath was taken, the tongue dropped and a wooden tongue

depressor was inserted deep into the oral cavity. A sterile swab was rolled across the laryngo-pharynx and placed into AMIES medium without charcoal. Rectal swabs were collected by lifting the pup and spreading the hind flippers. A sterile swab was inserted approximately 7 cm into the rectum, rolled against the rectal wall, removed, and placed into AMIES medium without charcoal. There was no laboratory on St. George Island; therefore, on 19 August 1991, swabs in transport medium from the North, Zapadni, and East rookeries were transported to St. Paul Island and streaked onto primary isolation media. Maximum time between sampling and primary isolation was 72 hours for samples from St. George Island. Swabs from St. Paul and Medny Islands were streaked onto primary isolation media the same day they were taken.

C. Isolation of Bacteria

Chocolate II Agar (BBL, Maryland, USA) plates were inoculated with ocular swabs of pups from all rookeries. Trypticase Soy Agar with 5% Sheep Blood (TSA II; BBL, Maryland, USA) plates were inoculated with pharyngeal and rectal swabs from St. George Island. MacConkey II Agar (BBL, Maryland, USA) and Hektoen Enteric Agar (BBL, Maryland, USA) plates were inoculated with rectal swabs from all locations and broken-off into Selenite-F Broth (BBL, Maryland, USA), incubated for 12 to 18 hours at 37° Celsius, and a loopful of the broth was streaked onto Hektoen Enteric Agar (BBL, Maryland, USA). Culture plates were incubated for 18 to 24 hours at 33 to 37° Celsius and examined for bacterial growth. Colony shape and pigmentation was described, as were zones of hemolysis and changes in color of the media. Colony types were inoculated onto Chocolate II, TSA II, or nutrient agar slants (BBL, Maryland, USA), incubated for 18 to 24 hours at 33 to 37° Celsius, and stored at 2 to 8° Celsius. On 1 September 1990 swabs, culture plates, and slants from the Pribilof Islands were transported to Moss Landing Marine Laboratories

and refrigerated (2 to 8° Celsius). Culture plates from Medny Island were incubated at 25 to 34° Celsius for 24 hours and maintained at 2 to 8° Celsius until shipment to Moss Landing Marine Laboratories on 8 September 1990.

D. Identification of Bacteria

Bacteria from the Chocolate II, TSA II, and nutrient agar slants were plated onto TSA II (BBL, Maryland, USA) and MacConkey II Agar (BBL, Maryland, USA) plates to observe hemolysis (production of clear, semi-clear, or greening zone in the media), swarming (growth in waves on media), colony pigmentation (color), and colony morphology (shape). Bacteria were stained using Gram reagents (BBL, Maryland, USA), and observed under a compound microscope to determine Gram reaction and cellular morphology. Gram negative rods were identified using the Minitek Miniaturized Differentiation System for the Identification of Enterobacteriaceae (BBL, Maryland, USA). Gram negative isolates were tested for possession of oxidase by placing Taxo N discs (BBL, Maryland, USA) on the MacConkey II Agar plates inoculated with bacteria, and the media was examined for a color change. Motility was determined by observing movement of bacteria in a hanging drop slide under a compound microscope. Bacterial cultures identified biochemically as Salmonella enteritidis were subsequently screened with the Salmonella Grouping Serum Set (BBL, Maryland, USA) to observe clumping. Positive reactions (clumps formed in solution on a slide) were then tested with group-specific (A-E) Salmonella antiserum (BBL, Maryland, USA). Three of 23 Salmonella cultures were randomly selected and submitted to Pathology Laboratory (Los Gatos, CA, USA) for independent identification.

Production of catalase was determined by adding bacteria to a drop of 3% hydrogen peroxide on a glass slide. Catalase positive (production of bubbles) cultures were tested for clumping factor by adding a drop of rabbit plasma to a suspension of bacteria on a glass slide

and observed for clumping. Cultures that tested negative for bound clumping factor were used to inoculate tubes of rabbit plasma and incubated 4 to 24 hours at 37°C to observe clotting. Bacitracin discs (BBL, Maryland, USA) were placed on TSA II (BBL, Maryland, USA) inoculated with gram positive cocci (hemolytic, catalase negative) to observe inhibition of growth (Bacitracin sensitivity). A CAMP test was done by streaking a test organism across a TSA II (BBL, Maryland, USA) plate inoculated with β -toxin producing Staphylococcus aureus (BBL, Maryland, USA), and observed for an enhanced hemolyzed zone. Gram positive cocci were identified using the Minitek Miniaturized Differentiation System for the Identification of Gram Positive Cocci (BBL, Maryland, USA). Standard methods for identifying aerobic bacteria were followed (Jang et al. 1990).

E. Statistical Analysis

One way analysis of variance (ANOVA) was used to determine if there was a significant difference among rookeries of the Pribilof Islands in mean weight of males and females. Also, a Student's T-test (t-test) was used to determine if there was a significant difference between St. Paul and St. George Islands in mean weight of males and females. A Chi Square test was used to determine if there was a significant difference in frequencies of occurrence of species of pathogenic bacteria among rookeries, and number of males and females with a pathogenic bacterium. T-tests were used to determine if there was a significant difference in mean weight and mean index of condition for males and females with and without a pathogenic bacterium. An ANOVA was used to determine if there was a significant difference among rookeries in mean number of bacterial species and mean number of species of pathogenic bacteria per pup.

Thirty pups of St. Paul, St. George, and Medny Islands were sub-sampled randomly, and ANOVA was used to determine if there was a significant difference among

islands in mean number of bacterial species and mean number of species of pathogenic bacteria per pup. A linear regression was used to determine if pup mortality (number of dead pups/number of live pups X 100; York and Fowler pers. com. 1990) and mean numbers of bacterial species and mean numbers of species of pathogenic bacteria per pup, and frequencies of occurrence of species of pathogenic bacteria were related for rookeries of the Pribilof and Medny Islands. Swartzman et al. (unpl. data 1987) reported few differences in pup density among rookeries, therefore, a linear regression was used to determine if number of live pups (York and Fowler pers. com. 1990), and mean numbers of bacterial species and mean numbers of species of pathogenic bacteria per pup, and frequencies of occurrence of species of pathogenic bacteria were related for rookeries of the Pribilof and Medny Islands. Also, a linear regression was used to determine if there was a relationship between prevalence (%) of group-D Salmonella and pup mortality (%) for the Vostochni, Gorbatch, Little Zapadni, Zapadni, and Zapadni Reef rookeries of St. Paul Island. A linear regression was used to determine if weight and index of condition of pups (Pribilof Islands only) and number of bacterial species, number of species of pathogenic bacteria per pup, and frequency of occurrence of species of pathogenic bacteria were related for rookeries of the Pribilof and Medny Islands. Frequency distributions were used to determine deviation from normality, and homogeneity of variance was tested using Cochran's test (Winer 1971). A Ryan-Einot-Gabriel-Welsch Q-test was used for posteriori multiple comparisons (Day and Quinn 1989). A 0.05 level of significance was used to test the null hypothesis for all statistical tests.

RESULTS

Pup weight was correlated positively with standard length, cranial width, index of fat thickness, and fore-flipper length for pups of the Pribilof Islands, and with standard length for pups of Medny Island (Table 1). There was no significant difference among rookeries of

St. Paul, St. George, and Medny Islands in mean weight of male and female pups (Table 2). There was no significant difference between St. Paul and St. George Islands in mean weight of males and females. Therefore, males and females of the Pribilof Islands were combined and tested with pups of Medny Island. There was no significant difference between the Pribilof and Medny Islands in mean weight of male and female pups (Table 2).

Thirty-two taxa of bacteria were identified from ocular, pharyngeal, and rectal swabs (Table 3). Coliform (Escherichia, Klebsiella, Enterobacter, and Citrobacter) and Proteus were the most common bacteria (Tables 4, 5, and 6). Five taxa of bacteria have been reported as pathogenic in Otariids (Table 7), and thus, were used for analysis of the effect of a pathogenic bacterium on pup weight and index of condition. Pups on the Reef rookery of St. Paul Island had the greatest number of species of pathogenic bacteria, and pups on the North and Zapadni rookeries of St. George Island the least ($\chi^2 = 45.223$, $P = 0.0001$).

Group-D Salmonella was isolated from rectal swabs of pups on 5 rookeries of St. Paul Island only. Prevalence (%) of group-D Salmonella was greater in female pups ($\chi^2 = 5.261$, $P = 0.0218$) than male pups. Prevalence (%) of group-D Salmonella was correlated positively with pup mortality (%) for the Little Zapadni, Zapadni, Zapadni Reef, Vostochni, and Gorbach rookeries ($r^2 = 0.917$, $P = 0.0105$; Fig. 2). Mean weight (5 of 10 comparisons) and mean index of condition (3 of 10 comparisons) of males and females with a species of pathogenic bacterium isolated from rectal swabs was significantly less than mean weight and mean index of condition of male and female pups without a species of pathogenic bacterium (Table 8 and 9).

Pseudomonas aeruginosa was isolated from rectal, ocular, or pharyngeal swabs of pups on all rookeries of St. Paul Island, except the Zapadni rookery, from six pups on the East Cliffs rookery and one pup on the North rookery of St. George Island, and from three pups on the Urilie rookery of Medny Island (Tables 4, 5, and 6). There was no significant

difference in frequency of isolation of Pseudomonas aeruginosa from rectal swabs of males and females ($\chi^2=0.133$, $P= 0.715$). Acinetobacter lwoffii was isolated from rectal, ocular, or pharyngeal swabs of pups on eight rookeries of St. Paul Island, and the East Cliffs and North rookeries of St. George Island (Tables 4, 5, and 6). Beta-hemolytic Streptococcus sp. and Staphylococcus aureus were isolated from ocular or pharyngeal swabs of pups on all rookeries (Tables 5 and 6).

Mean number of bacterial species per pup was significantly greater for pups on the Little Zapadni, Reef, Vostochni, Zapadni, and Morjovi rookeries, and mean number of species of pathogenic bacteria per pup was significantly greater for pups on the Reef rookery (Table 10). Comparisons among islands indicated mean number of bacterial species and mean number of species of pathogenic bacteria per pup were significantly greater for pups of St. Paul Island (Table 11) than Medny Island, but not St. George Island.

Linear regressions between pup mortality (%) and number of live pups, and number of bacterial species and species of pathogenic bacteria were not significant (Table 12). Mean weight of pups was correlated positively with mean number of species of pathogenic bacteria per pup for rookeries of the Pribilof Islands and Medny Island (Table 13). The index of condition was correlated positively with number of bacterial species and number of species of pathogenic bacteria for pups of the Pribilof Islands (Table 13).

DISCUSSION

Comparisons between pup weight and total length, cranial width, index of fat thickness, and fore-flipper length were significant, but regression coefficients were low. This indicated sample size was adequate but variability in morphometric measurements was great. Pups were difficult to restrain, work was fast-paced, and weather was often harsh, all perhaps contributing to variability. There was no significant difference in mean weight of

pups among rookeries and between the Pribilof and Medny Islands, indicating pups probably were of similar age at time of sampling. I believe the best measure of pup health was the index of condition because it incorporated weight, standard length, and the index of fat thickness. Ultra-sonic measurement of fat thickness (Gales and Burton 1987) may be a more reliable measure than the index of fat thickness, but this was not possible in the field due to the great number of pups sampled.

Coliform and Proteus were the most common bacteria in pharyngeal, ocular, and rectal swabs, which is not surprising because pinniped rookeries are contaminated heavily with feces. Mouths of pups contained fecal material, and obtaining uncontaminated pharyngeal swabs was difficult. Overall growth of bacteria was confluent on blood agar, and many beta-hemolytic colonies may have been missed. Pharyngeal swabs did not appear useful for the isolation of respiratory pathogens because of confluent growth of fecal contaminants.

Coliform bacteria were less abundant in ocular swabs than pharyngeal swabs, indicating eyes of pups were less contaminated with fecal material than mouths. Staphylococcus sp., Streptococcus sp., and Pseudomonas aeruginosa were isolated from ocular swabs, which may explain the presence of pups with exudate around eyes. Ocular infection with Pseudomonas aeruginosa, Staphylococcus, Streptococcus, and Neisseria is common in juvenile seals (Laukner 1985). In 1987, no diagnosis of ocular infection was made in pups of northern fur seals (Spraker unpl. data 1987). Therefore, the nictitating membrane of pups may be an efficient primary barrier to coliform, staphylococcal, streptococcal, and pseudomonad infection.

Escherichia coli was the most common bacteria in rectal swabs, and probably was part of the normal flora. Hookworm infection of pups was diagnosed at necropsy (Spraker unpl. data 1987) and may allow Coliform and Proteus to affect pups. An hemolytic E. coli

was cultured from my ocular swabs of pups on the Zapadni, Lukanin, and Tolstoi rookeries and from lungs of dead pups (Spraker pers. comm. 1991). This indicated hemolytic Escherichia coli may be an important pathogen in pups.

Five taxa of bacteria appeared to affect pup weight and index of pup condition. Pseudomonas sp. are distributed widely in soil and water (Washington 1985), and were isolated from pharyngeal and ocular swabs on almost all rookeries of the Pribilof and Medny Islands. Pseudomonas aeruginosa may be responsible for seal epizootics (Rand 1975) and is a frequent cause of bacteremia and pneumonia, especially in an immuno-suppressed host (Washington 1985). P. aeruginosa was isolated from rectal swabs on six of 16 rookeries only, indicating P. aeruginosa may not be part of the normal intestinal flora of northern fur seal pups. Recovery of P. aeruginosa from male and female pups was not significantly different, indicating males and females encountered P. aeruginosa with similar frequency.

P. aeruginosa appeared to affect male pups only. Males may grow faster and have greater nutritional requirements than females (Costa and Gentry 1986). Infection of males by P. aeruginosa may depress growth rates by debilitating pups, resulting in significantly less weight and index of condition. Also, males may be more active than females, and consequently, experience more trauma from adults. This may increase prevalence (%) of P. aeruginosa infection.

Acinetobacter lwoffii was isolated from rectal swabs of pups on the Reef rookery only, indicating A. lwoffii also may not be part of the normal intestinal flora of northern fur seal pups. Acinetobacter sp. were isolated from dead adult southern fur seals with enteritis (Baker and McCann 1989), and appeared to affect condition of male pups only. The behavioral differences between males and females discussed above may have increased exposure of male pups to A. lwoffii. Acinetobacter sp. are free-living bacteria, which may explain the greater prevalence of A. lwoffii in pharyngeal and ocular swabs than in rectal

swabs. A. lwoffii was isolated from rectal samples of apparently healthy northern fur seal pups of St. Paul Island (Keyes et al. 1981), but did not appear to affect condition of female pups. This indicated A. lwoffii probably was not pathogenic under normal circumstances.

Staphylococcus aureus was distributed among pups on most rookeries and probably did not affect growth of pups. S. aureus is the etiological agent of staphylococcal enterocolitis (Jang et al. 1990), but determining the importance of S. aureus as an enteric pathogen would require a greater number of swabs and diagnosis of pups.

S. aureus was the most common species of Staphylococcus isolated from pharyngeal swabs, and is capable of causing respiratory disease in pinnipeds (Sweeney and Gilmartin 1974). At necropsy, Spraker (unpl. data 1987) diagnosed pneumonia in 8.9% of pups on St. Paul Island, and the prevalence (%) in pharyngeal swabs indicated S. aureus should be investigated as an etiological agent of pneumonia. Pasteurella sp. was isolated from terrestrial mammals with respiratory disease (Snipes et al. 1980), and from pups of northern fur seals (Keyes et al. 1968). Pasteurella sp. was not isolated from my swabs but should not be dismissed as an etiological agent of pneumonia.

S. aureus was common in ocular swabs, and conjunctivitis has been diagnosed in pups (Spraker pers. com. 1990). The prevalence of S. aureus in ocular swabs indicated it should be considered potentially pathogenic. Cultures from live and freshly dead pups with conjunctivitis may determine the importance of S. aureus as an ocular pathogen. No attempt was made to culture Chlamydia sp. from my ocular swabs and Chlamydia sp. may be an important ocular pathogen (Spraker pers. com. 1990)

Beta-hemolytic Streptococcus sp. (Streptococcus pyogenes and Streptococcus agalactiae) were infrequently isolated from pharyngeal and ocular swabs on almost all rookeries, and are pathogenic in pinnipeds (Laukner 1985). Streptococcus agalactiae is an

important source of bovine mastitis, and affects mammary glands (Jang et al. 1990).

Cultures of mammary glands from lactating females and mouths of pups may determine if S. agalactiae is transmitted from mother to pup during suckling. Infection of wounds by Streptococcus sp. is common in pinnipeds (Laukner 1985). Secondary bacterial infection of traumatic wounds was an important source of mortality for Antarctic fur seals (Baker and McCann 1989) and pups of northern fur seals (Keyes et al. 1975). The under-developed immune system in young animals (Baker and McCann 1989) and the prevalence (%) of Streptococcus sp. may make pups susceptible to infection. Pneumococci have been identified as a major source of mortality in pinnipeds (Laukner 1985), but were not isolated in my swabs; their effect on northern fur seal pup mortality is unknown.

The most significant finding was group-D Salmonella in rectal swabs of pups on five rookeries of St. Paul Island. Stroud and Roelke (1980) isolated Salmonella enteritidis from two pups with a meningoencephalomyelitis of 175 dead pups examined on St. George Island. Keyes et al. (1981) reported no Salmonella sp. from dead pups of northern fur seals of St. Paul Island. Gilmartin et al. (1979) reported prevalence (33%) of several Salmonella serotypes in apparently healthy pups of northern fur seals of the Channel Islands, and suggested Salmonella caused mild gastroenteritis only. In 1990, prevalence (%) of group-D Salmonella on the Little Zapadni (20%) and Zapadni Reef (26%) rookeries of the Pribilof Islands were comparable to Gilmartin's study. Prevalence (%) of group-D Salmonella was correlated positively with pup mortality (%) for the Little Zapadni, Zapadni, Zapadni Reef, Vostochni, and Gorbach rookeries. Thus, group-D Salmonella may have affected pup mortality (%) on these rookeries before sampling.

Mean weight of males and females with group-D Salmonella was significantly less than males and females without group-D Salmonella, indicating group-D Salmonella may have affected weight gain of pups. Nutritionally-stressed pups may be more susceptible to

infection by group-D Salmonella, and pup mortality may be greater in pups of lower weight with group-D Salmonella. Prevalence (%) of group-D Salmonella was greater in female pups. Females are generally smaller in size than males (Lander 1979) and may not be as active. If females remain in contact with sources of group-D Salmonella for a greater amount of time than males, they may be more vulnerable to infection. Group-D Salmonella appeared to affect the index of condition for females only, indicating group-D Salmonella may have a greater effect on the growth of females than males. Males may enter water before females, and this may wash sources of group-D Salmonella from male pups. It is interesting three of five rookeries where group-D Salmonella was isolated are located in a bay used frequently by large fishing vessels. If sources of group-D Salmonella are discharged from these vessels and wash ashore, pups may become infected.

Calambokidis and Gentry (1985) reported tagged pups that died weighed less at birth than the weight at birth of the total marked population, and pups that died of infectious disease weighed less at birth than the average weight at birth. In 1990, mean weight (5 of 10 comparisons) and index of condition (3 of 10 comparisons) of my pups with a pathogenic bacterium were significantly less than pups without these pathogenic bacteria. Weight and index of condition of pups with pathogenic bacteria may have been affected by timing and susceptibility to infection. Infection of pups with relatively normal weight and condition at birth may prevent weight gain and growth. Alternatively, pups of relatively low birth weight and condition may be more susceptible to infection, and pups of lower weight and condition with pathogenic bacteria may have greater mortality than pups without these bacteria. The index of condition seemed less sensitive to the effects of bacteria, indicating most pathogenic bacteria may have affected weight and not growth.

Number of bacterial species and number of species of pathogenic bacteria per pup increased with the index of condition (Pribilof Islands only), and not weight of pups. Large

pups may be more mobile than small pups, and behavioral differences may have exposed larger pups to a greater number of species of bacteria. The positive slopes of the regression lines indicated most bacteria did not affect the health of pups at time of sampling.

Comparisons among rookeries indicated pups on the Little Zapadni, Zapadni, Reef, Vostochni, and Morjovi rookeries of St. Paul Island had a significantly greater mean number of bacterial species and the Gorbach and Kitovi rookeries of St. Paul Island the least. Number of live pups were relatively greater for the Little Zapadni, Zapadni, Reef, and Vostochni rookeries, but did not appear related to mean number of bacterial species per pup. Thus, bacterial diversity (number of species per pup) was not affected by number of live pups on rookeries. Although, there was no significant relationship between pup mortality (%) and mean number of bacterial species per pup, pup mortality (%) on the Little Zapadni, Zapadni, and Reef rookeries was greater than the average pup mortality (%) of St. Paul Island, and pup mortality (%) on the Kitovi and Gorbach rookeries was less than the average pup mortality (%) of St. Paul Island.

Mean number of species of pathogenic bacteria per pup and frequency of occurrence of species of pathogenic bacteria were significantly greater for the Reef rookery of St. Paul Island and the East rookery of St. George Island. Pup mortality (%) for the Reef rookery was greater than the average pup mortality (%) of St. Paul Island, but did not appear related to mean number of species of pathogenic bacteria per pup, or frequency of occurrence of species of pathogenic bacteria.

Comparisons among islands indicated pups on St. Paul Island had a greater mean number of bacterial species per pup and mean number of species of pathogenic bacteria per pup than Medny Island, but not St. George Island. Pups on islands with a greater number of animals (St. Paul Island) may have been exposed to a greater number of bacteria. The greater mean number of bacterial species and mean number of species of pathogenic bacteria per pup

for St. Paul Island, may have contributed to the greater average pup mortality (%) of St. Paul Island than St. George Island and Medny Island.

SUMMARY

Most bacteria isolated from pups of northern fur seals could be capable of causing disease in a compromised host, but many pups appeared healthy at time of sampling. Therefore, bacteria probably did not affect most pups. Pups become stressed nutritionally, traumatized, and exposed to extremely harsh weather which may have allowed bacteria to affect the health of pups after sampling. Few bacteria isolated were reported etiological agents of mass pinniped deaths, and for the Pribilof Islands, numbers of pathogenic species of bacteria and pup mortality (%) were not related. Pseudomonas aeruginosa, Acinetobacter lwoffii, Streptococcus pyogenes, Streptococcus agalactiae, Staphylococcus aureus, Escherichia coli-hemolytic, and group-D Salmonella were isolated from pups and probably are associated with morbidity and mortality. Prevalence (%) of group-D Salmonella was greatest for pups on the Little Zapadni, Zapadni, and Zapadni Reef rookeries of St. Paul Island, and may have contributed to greater pup mortality for these rookeries than the average island mortality. Diagnosis of pups on the Reef, Zapadni, Zapadni Reef, and Little Zapadni rookeries of St. Paul Island would be valuable and for these rookeries and pup mortality should be examined in the future.

Comparisons among rookeries indicated pups on the Little Zapadni, Zapadni, Reef, Vostochni, and Morjovi rookeries of St. Paul Island had a significantly greater mean number of bacterial species and the Gorbach and Kitovi rookeries of St. Paul Island the least. Number of live pups was relatively greater for the Little Zapadni, Zapadni, Reef, and Vostochni rookeries, but did not affect bacterial diversity. Although there was no significant relationship between pup mortality (%) and mean number of bacterial species per pup, pup

mortality (%) on the Little Zapadni, Zapadni, and Reef rookeries was apparently greater than the average pup mortality of St. Paul Island. Pup mortality (%) on the Kitovi and Gorbach rookeries was apparently less than the average pup mortality (%) of St. Paul Island.

Pups on St. Paul Island had a significantly greater number of species of pathogenic bacteria than Medny Island but not St. George Island. The greater number of animals on St. Paul Island may represent a greater source of pathogenic bacteria. Pups of St. Paul Island had a significantly greater number of bacterial species and greater number of species of pathogenic bacteria which may contribute to the greater average island pup mortality (%).

Comparisons between pup mortality (%), number of live pups, weight and index of condition of pups, and number of bacterial species, and number of species of pathogenic bacteria were not significant, indicating most bacteria did not affect pup health and mortality. Bacteria may affect pup mortality when associated with traumatic, nutritional, or environmental stress. The wide distribution of bacteria in pups from all rookeries indicated pups are exposed to many of the same bacteria. The physiological state of pups may determine the effects of bacteria on pup health. Crowded, nutritionally-stressed pups may be more susceptible to invasion by pathogenic and opportunistically pathogenic bacteria, and pup mortality may be greater in pups of lower weight infected with a pathogenic bacteria. At this time, differential pup mortality (%) among most rookeries cannot be explained by bacteria isolated from pups.

This survey of bacteria indicated group-D Salmonella was the most important potential pathogen. Prevalence (%) of group-D Salmonella was correlated positively with pup mortality (%) for the Little Zapadni, Zapadni, Zapadni Reef, Vostochni, and Gorbach rookeries, indicating group-D Salmonella may increase pup mortality. Female pups appeared affected by group-D Salmonella more than male pups. If group-D Salmonella increases mortality of female pups, fewer pups would be born in subsequent years, which may prevent

increases in numbers of northern fur seals on the Little Zapadni, Zapadni, Zapadni Reef, Vostochni, and Gorbach rookeries of St. Paul Island.

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Table 1. Linear regressions of weight (kg) with standard length (cm), cranial width (cm), index of fat thickness (cm), and fore-flipper length (cm) for northern fur seal pups on the Pribilof Islands and Medny Island.

	n	REGRESSION COEFFICIENT	F VALUE	P VALUE
ST. PAUL				
<u>MALES AND FEMALES</u>				
STANDARD LENGTH	360	0.754	471.035	0.0001
CRANIAL WIDTH	360	0.609	210.515	0.0001
FAT PINCH	360	0.466	99.447	0.0001
FORE-FLIPPER LENGTH	360	0.577	178.937	0.0001
<u>MALES</u>				
STANDARD LENGTH	180	0.732	205.254	0.0001
CRANIAL WIDTH	180	0.569	85.117	0.0001
FAT PINCH	180	0.527	68.357	0.0001
FORE-FLIPPER LENGTH	180	0.534	70.875	0.0001
<u>FEMALES</u>				
STANDARD LENGTH	180	0.657	135.057	0.0001
CRANIAL WIDTH	180	0.511	62.873	0.0001
FAT PINCH	180	0.412	36.424	0.0001
FORE-FLIPPER LENGTH	180	0.474	51.529	0.0001
ST. GEORGE				
<u>MALES AND FEMALES</u>				
STANDARD LENGTH	90	0.662	68.644	0.0001
CRANIAL WIDTH	90	0.463	24.034	0.0001
FAT PINCH	90	0.449	22.161	0.0001
FORE-FLIPPER LENGTH	90	0.166	2.487	0.1184
<u>MALES</u>				
STANDARD LENGTH	45	0.678	35.79	0.0001
CRANIAL WIDTH	45	0.481	12.543	0.0001
FAT PINCH	45	0.517	15.327	0.0003
FORE-FLIPPER LENGTH	45	0.052	0.116	0.7354
<u>FEMALES</u>				
STANDARD LENGTH	45	0.505	15.072	0.0003
CRANIAL WIDTH	45	0.271	3.486	0.0686
FAT PINCH	45	0.395	8.112	0.0067
FORE-FLIPPER LENGTH	45	0.445	10.876	0.0019
MEDNY				
<u>MALES AND FEMALES</u>				
STANDARD LENGTH	31	0.888	108.047	0.0001
<u>MALES</u>				
STANDARD LENGTH	16	0.865	38.655	0.0001
<u>FEMALES</u>				
STANDARD LENGTH	15	0.892	54.436	0.0001

Table 2. Comparison of mean weight (kg) of males and females among rookeries using ANOVA, and between islands using Student's t-test, for northern fur seal pups of St. Paul, St. George, and Medny Islands.

	n	TEST STATISTIC	P VALUE
AMONG ROOKERIES OF ST. PAUL			
MALES	180	F= 0.711	0.7267
FEMALES	180	F= 1.392	0.1806
AMONG ROOKERIES OF ST. GEORGE			
MALES	45	F= 2.965	0.0625
FEMALES	45	F= 1.768	0.1832
BETWEEN ST. PAUL AND ST. GEORGE			
MALES	225	t= 1.061	0.2897
FEMALES	225	t= 0.305	0.7605
BETWEEN PRIBILOF AND MEDNY			
MALES	240	t= 0.693	0.4889
FEMALES	240	t= 0.658	0.5111

Table 3. Bacteria isolated from swabs of northern fur seal pups of the Pribilof Islands and Medny Island.

BACTERIUM	TYPE OF SWAB		
	RECTAL	OCULAR	PHARYNGEAL
<u>Acinetobacter lwoffii</u>	X	X	X
<u>Citrobacter freundii</u>	X	X	X
<u>Enterobacter cloacae</u>	X	X	X
<u>Enterobacter aerogenes</u>	X	X	X
<u>Escherichia coli</u>	X	X	X
<u>Hafnia alvei</u>	X		
<u>Klebsiella oxytoca</u>	X	X	X
<u>Micrococcus sp.</u>		X	X
<u>Morganella morganii</u>	X		
<u>Proteus mirabilis</u>	X	X	X
<u>Proteus vulgaris</u>	X	X	X
<u>Pseudomonas aeruginosa</u>	X	X	X
Group-D <u>Salmonella</u>	X		
<u>Serratia marcescens</u>	X		
<u>Staphylococcus aureus</u>	X	X	X
<u>Staphylococcus chonii</u>		X	
<u>Staphylococcus epidermidis</u>		X	X
<u>Staphylococcus hyicus</u>		X	X
<u>Staphylococcus simulans</u>		X	
<u>Staphylococcus warneri</u>		X	X
<u>Staphylococcus xylosus</u>		X	X
<u>Streptococcus acidominimus</u>		X	X
<u>Streptococcus agalactiae</u>		X	X
<u>Streptococcus avium</u>		X	X
<u>Streptococcus bovis</u>		X	X
<u>Streptococcus faecalis</u>		X	X
<u>Streptococcus mitis</u>		X	X
<u>Streptococcus morbillorum</u>		X	
<u>Streptococcus mutans</u>		X	X
<u>Streptococcus pyogenes</u>	X	X	X
<u>Streptococcus sanguis</u>		X	
<u>Streptococcus uberis</u>		X	X

Table 4. Percentage of northern fur seal pups (n= 30/rookery) with bacterial taxa isolated from rectal swabs of pups of the Pribilof Islands and Medny Island.

BACTERIUM	ST. PAUL ISLAND											ST. GEORGE ISLAND			MEDNY ISLAND	
	ISLAND											ISLAND			ISLAND	
	V	M	LZ	Z	ZR	L	P	PC	K	T	R	G	N	E	Z	U
<u>group-D Salmonella</u>	7	0	20	13	27	0	0	0	0	0	0	10	0	0	0	0
<u>Pseudomonas aeruginosa</u>	0	37	0	0	0	23	10	10	0	0	17	0	0	20	0	0
<u>Acinetobacter lwoffii</u>	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0
<u>Staphylococcus aureus</u>	*	*	*	*	*	*	*	*	*	*	*	*	23	0	0	*
<u>Streptococcus pyogenes</u>	*	*	*	*	*	*	*	*	*	*	*	*	7	20	0	*
<u>Citrobacter freundii</u>	13	40	37	43	30	20	40	10	20	13	13	3	27	10	20	13
<u>Enterobacter sp.</u>	27	7	27	17	17	0	10	20	7	23	13	3	10	7	7	17
<u>Klebsiella oxytoca</u>	13	0	7	13	0	10	10	3	0	0	0	0	17	7	0	3
<u>Escherichia coli</u>	77	47	73	83	60	37	53	67	30	53	77	60	37	70	47	57
<u>Proteus sp.</u>	27	30	33	33	13	0	23	10	0	27	20	20	30	17	27	7
<u>Serratia marcescens</u>	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0
<u>Morganella morganii</u>	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0
<u>Hafnia alvei</u>	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0

ROOKERIES: V= Vostochni, M= Morjovi, LZ= Little Zapadni, Z= Zapadni, ZR= Zapadni Reef, L= Lukanin, P= PolovinaPC= Polovina Cliff, K= Kitovi, T= Tolstoi, R= Reef, G= Gorbach, N= North, E= East Cliffs, U= Urtlie

* = swabs were not used to inoculate plates of TSA + %5 Sheep Blood

Table 5. Percentage of northern fur seal pups (n= 30/rookery) with bacterial taxa isolated from ocular swabs of pups of the Pribilof Islands and Medny Island.

BACTERIUM	ST. PAUL												ST. GEORGE			MEDNY	
	ISLAND												ISLAND			ISLAND	
	V	M	LZ	Z	ZR	L	P	PC	K	T	R	G	N	E	Z	U	U
<u>Staphylococcus aureus</u>	7	7	13	13	7	0	3	0	7	3	13	0	0	0	3	10	
<u>Staphylococcus epidermidis</u>	7	10	20	10	7	7	10	10	0	20	3	3	13	0	27	13	
<u>Staphylococcus xylosum</u>	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<u>Staphylococcus warneri</u>	0	0	3	0	0	0	3	3	3	0	0	0	0	0	0	0	
<u>Staphylococcus hyicus</u>	0	0	0	3	3	0	0	0	10	0	3	3	0	0	0	0	
<u>Staphylococcus simulans</u>	3	0	7	0	0	0	0	0	0	3	3	0	0	0	3	0	
<u>Staphylococcus cohnii</u>	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	7	
<u>Micrococcus sp.</u>	7	30	27	13	10	10	0	17	0	17	7	0	0	17	20	3	
<u>Streptococcus pyogenes</u>	10	0	0	7	7	0	0	3	0	0	0	0	3	3	3	3	
<u>Streptococcus agalactiae</u>	0	0	10	3	0	7	3	3	0	0	0	0	7	7	0	0	
<u>Streptococcus faecalis</u>	20	10	10	10	3	0	0	7	0	27	3	3	3	3	13	10	
<u>Streptococcus mutans</u>	0	0	7	7	0	3	10	0	0	0	0	0	0	3	13	10	
<u>Streptococcus bovis</u>	0	0	0	0	10	3	3	0	3	0	0	0	0	0	0	0	
<u>Streptococcus acidominimus</u>	0	3	0	0	0	0	0	0	0	0	0	0	0	3	7	0	
<u>Streptococcus avium</u>	3	0	0	0	0	0	3	0	3	0	3	10	0	0	0	0	
<u>Streptococcus mitis</u>	7	3	3	7	0	0	3	0	0	0	3	0	0	0	0	0	
<u>Streptococcus uberis</u>	17	0	0	0	0	0	0	3	0	7	3	0	0	0	13	0	
<u>Streptococcus morbillorum</u>	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	13	
<u>Streptococcus sanguis</u>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	
<u>Acinetobacter lwoffii</u>	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	0	
<u>Pseudomonas aeruginosa</u>	20	0	0	0	17	13	10	10	7	10	0	7	0	0	0	10	
<u>Escherichia coli- hemolytic</u>	0	0	0	10	0	7	0	0	0	13	0	0	0	0	0	0	
<u>Escherichia coli</u>	20	37	17	20	13	17	17	7	23	10	13	10	97	70	53	27	
<u>Enterobacter sp.</u>	0	3	3	7	3	0	0	3	0	0	7	3	0	0	20	0	
<u>Citrobacter freundii</u>	7	0	0	3	3	0	0	0	0	0	3	0	0	0	0	3	
<u>Klebsiella sp.</u>	0	3	0	3	3	0	0	3	7	0	3	0	0	0	0	0	
<u>Proteus sp.</u>	17	7	33	10	10	20	7	3	10	3	20	3	7	37	7	10	

ROOKERIES: V= Vostochni, M= Morjovi, LZ= Little Zapadni, Z= Zapadni, ZR= Zapadni Reef, L= Lukanin, P= Polovina, PC= Polovina Cliff, K= Kitovi, T= Tolstoi, R= Reef, G= Gorbach, N= North, E= East, U= Uriilie

Table 6. Percentage of northern fur seal pups (n= 30/rookery) with bacterial taxa isolated from pharyngeal swabs of pups of the Pribilof Islands and Medny Island.

ROOKERIES OF:	ST. PAUL												ST. GEORGE			MEDNY	
	ISLAND												ISLAND			ISLAND	
	V	M	LZ	Z	ZR	L	P	PC	K	T	R	G	N	E	Z	U	U
<u>BACTERIUM</u>	23	7	17	13	13	13	17	13	20	13	23	23	7	20	13		17
<u>Staphylococcus aureus</u>																	
<u>Staphylococcus epidermidis</u>	10	7	13	17	7	17	10	13	37	13	23	10	7	7	13		10
<u>Staphylococcus xylosus</u>	0	0	0	0	7	0	0	0	0	0	0	0	0	0	0		0
<u>Staphylococcus warneri</u>	0	0	0	0	0	0	0	0	7	0	0	7	0	0	0		0
<u>Staphylococcus hyicus</u>	7	0	0	0	0	0	3	0	3	0	3	0	0	0	3		7
<u>Micrococcus sp.</u>	0	0	0	0	0	7	0	0	0	0	3	0	0	0	0		0
<u>Streptococcus pyogenes</u>	0	13	0	10	17	10	13	20	10	10	17	10	7	17	10		7
<u>Streptococcus agalactiae</u>	7	7	0	3	3	3	7	3	10	0	20	3	0	13	3		10
<u>Streptococcus faecalis</u>	27	23	20	27	13	27	23	23	30	0	43	20	3	27	23		0
<u>Streptococcus mutans</u>	0	0	0	3	0	0	0	0	0	13	0	10	0	10	0		0
<u>Streptococcus bovis</u>	3	7	7	3	3	0	3	0	7	13	0	0	7	3	0		7
<u>Streptococcus avium</u>	3	0	10	0	3	0	7	0	7	0	0	0	0	0	7		3
<u>Streptococcus mitis</u>	13	0	13	7	7	0	7	10	0	3	23	10	3	3	20		10
Coliform group	53	100	93	87	77	40	83	60	90	100	97	93	97	63	87		100
<u>Acinetobacter lwoffii</u>	0	20	20	17	17	0	10	3	0	0	0	0	7	3	0		0
<u>Pseudomonas aeruginosa</u>	20	0	7	0	3	17	0	10	23	10	30	7	3	0	0		0
<u>Proteus sp.</u>	13	40	27	0	17	33	0	10	17	33	30	23	20	13	10		20

ROOKERIES: V= Vostochni, M= Morjovi, LZ= Little Zapadni, Z= Zapadni, ZR= Zapadni Reef, L= Lukanin, P= Polovina, PC= Polovina Cliff, K= Kitovi, T= Tolstoi, R= Reef, G= Gorbatch, N= North, E= East, U= Uriilie

Table 7. Bacteria reported as pathogenic in Otariids.

BACTERIUM	ORGAN SYSTEM AFFECTED	REFERENCE
<u>Salmonella</u> sp.	gastrointestinal pulmonary	Jellison and Milner 1958, Sweeney and Gilmartin 1974
<u>Pseudomonas</u> sp.	pulmonary	Sweeney and Gilmartin 1974
<u>Staphylococcus aureus</u>	pulmonary	Sweeney and Gilmartin 1974
<u>Streptococcus</u> sp.	pulmonary	Baker and McCann 1989
<u>Acinetobacter lwoffii</u>	gastrointestinal, pulmonary	Baker and McCann 1989

Table 8. Comparison of mean weight (kg) of northern fur seal pups with and without a species of pathogenic bacterium isolated from rectal swabs using a Student's t-test.

BACTERIUM	SEX	MEAN WEIGHT		SD	P VALUE
		n	(kg)		
group-D <u>Salmonella</u>	male	6	7.46	1.35	0.0002
no group-D <u>Salmonella</u>	male	69	9.83	1.45	
group-D <u>Salmonella</u>	female	17	7.39	1.01	0.0026
no group-D <u>Salmonella</u>	female	58	8.58	1.46	
<u>Pseudomonas aeruginosa</u>	male	16	7.80	1.10	0.0001
no <u>Pseudomonas aeruginosa</u>	male	74	10.00	1.95	
<u>Pseudomonas aeruginosa</u>	female	19	8.32	1.04	0.2390
no <u>Pseudomonas aeruginosa</u>	female	71	8.05	1.41	
<u>Acinetobacter lwoffii</u>	male	4	8.31	1.39	0.0092
no <u>Acinetobacter lwoffii</u>	male	11	11.20	1.68	
<u>Acinetobacter lwoffii</u>	female	2	7.13	0.53	0.0689
no <u>Acinetobacter lwoffii</u>	female	13	8.94	1.25	
<u>Streptococcus pyogenes</u>	male	2	10.00	1.41	0.6941
no <u>Streptococcus pyogenes</u>	male	13	9.55	1.57	
<u>Streptococcus pyogenes</u>	female	7	8.32	1.21	0.9446
no <u>Streptococcus pyogenes</u>	female	23	8.36	1.24	
<u>Staphylococcus aureus</u>	male	4	9.69	2.15	0.3643
no <u>Staphylococcus aureus</u>	male	11	8.77	1.49	
<u>Staphylococcus aureus</u>	female	3	6.92	0.52	0.0362
no <u>Staphylococcus aureus</u>	female	12	8.65	1.23	

Table 9. Comparison of mean index of condition (kg) of northern fur seal pups with and without a species of pathogenic bacterium isolated from rectal swabs using a Student's t-test.

BACTERIUM	SEX	n	MEAN INDEX OF CONDITION (kg)	SD	P
group-D <u>Salmonella</u>	male	6	0.15	0.055	0.1204
no group-D <u>Salmonella</u>	male	69	0.19	0.072	
group-D <u>Salmonella</u>	female	17	0.14	0.052	0.0104
no group-D <u>Salmonella</u>	female	58	0.18	0.054	
<u>Pseudomonas aeruginosa</u>	male	16	0.13	0.038	0.0057
no <u>Pseudomonas aeruginosa</u>	male	74	0.19	0.090	
<u>Pseudomonas aeruginosa</u>	female	19	0.16	0.074	0.3998
no <u>Pseudomonas aeruginosa</u>	female	71	0.15	0.067	
<u>Acinetobacter lwoffii</u>	male	4	0.21	0.064	0.0288
no <u>Acinetobacter lwoffii</u>	male	11	0.30	0.069	
<u>Acinetobacter lwoffii</u>	female	2	0.16	0.039	0.1942
no <u>Acinetobacter lwoffii</u>	female	13	0.22	0.061	
<u>Streptococcus pyogenes</u>	male	2	0.14	0.024	0.8698
no <u>Streptococcus pyogenes</u>	male	13	0.14	0.049	
<u>Streptococcus pyogenes</u>	female	7	0.11	0.053	0.6194
no <u>Streptococcus pyogenes</u>	female	23	0.12	0.047	
<u>Staphylococcus aureus</u>	male	4	0.14	0.054	0.6451
no <u>Staphylococcus aureus</u>	male	11	0.13	0.044	
<u>Staphylococcus aureus</u>	female	3	0.10	0.043	0.1445
no <u>Staphylococcus aureus</u>	female	12	0.14	0.042	

Table 10. Comparison of mean number of bacterial species ($F=6.93$, $P=0.0001$) and mean number of species of pathogenic bacteria ($F=3.36$, $P=0.0001$) per pup among rookeries of the Pribilof Islands and Medny Island using an ANOVA and Ryan-Einot-Gabriel-Welsch Q-multiple comparison test. Means with the same letter are not different significantly.

ROOKERY	MEAN NUMBER OF BACTERIAL SPECIES	RYAN-EINOT- GABRIEL-WELSCH Q-GROUP
LITTLE ZAPADNI	4.667	A
REEF	4.300	AB
VOSTOCHNI	4.233	ABC
ZAPADNI	3.967	ABCD
MORJOVI	3.800	ABCDE
EAST CLIFFS (GEORGE)	3.533	BCDEF
ZAPADNI (GEORGE)	3.300	BCDEF
NORTH (GEORGE)	3.172	CDEF
POLOVINA	3.067	DEF
TOLSTOI	3.033	DEF
LUKANIN	2.967	DEF
ZAPADNI REEF	2.967	DEF
URILIE (MEDNY)	2.903	DEF
POLOVINA CLIFFS	2.867	DEF
KITTOVI	2.800	EF
GORBATCH	2.433	F

ROOKERY	MEAN NUMBER OF SPECIES OF PATHOGENIC BACTERIA	RYAN- EINOT- GABRIEL-WELSCH Q-GROUP
REEF	1.333	A
EAST CLIFFS (GEORGE)	1.033	AB
MORJOVI	0.967	ABC
VOSTOCHNI	0.900	ABC
ZAPADNI	0.867	ABC
LITTLE ZAPADNI	0.867	ABC
ZAPADNI REEF	0.867	ABC
LUKANIN	0.833	ABC
POLOVINA CLIFFS	0.700	ABC
KITTOVI	0.700	ABC
POLOVINA	0.667	BC
TOLSTOI	0.600	BC
GORBATCH	0.600	BC
URILIE (MEDNY)	0.484	BC
ZAPADNI (GEORGE)	0.367	BC
NORTH (GEORGE)	0.345	C

Table 11. Comparison of mean number of bacterial species ($F=3.84$, $P=0.0252$) and mean number of species of pathogenic bacteria ($F=4.82$, $P=0.0103$) per pup among St. Paul, St. George, and Medny Islands using ANOVA and Ryan-Einot-Gabriel-Welsch Q-multiple comparison test. Means with the same letter are not different significantly.

ISLAND	MEAN NUMBER OF BACTERIAL SPECIES	RYAN-EINOT- GABRIEL-WELSCH Q- GROUP
ST. PAUL	3.867	A
ST. GEORGE	3.300	AB
MEDNY	2.967	B

ISLAND	MEAN NUMBER OF SPECIES OF PATHOGENIC BACTERIA	RYAN-EINOT- GABRIEL-WELSCH Q- GROUP
ST. PAUL	1.100	A
ST. GEORGE	0.700	AB
MEDNY	0.500	B

Table 12. Linear regressions of pup mortality (%) and number of live pups with mean number of bacterial species and mean number of species of pathogenic bacteria per pup, and frequency of occurrence of species of pathogenic bacteria for rookeries of the Pribilof Islands.

	REGRESSION COEFFICIENT	F VALUE	P VALUE
PRIBILOF ISLANDS			
ROOKERY MORTALITY WITH:			
MEAN NUMBER OF BACTERIAL SPECIES	0.112	0.126	0.7296
MEAN NUMBER OF SPECIES OF PATHOGENIC BACTERIA	0.205	0.437	0.5237
FREQUENCY OF OCCURRENCE OF SPECIES OF PATHOGENIC BACTERIA	0.307	1.038	0.3324
ST. PAUL ISLAND			
NUMBER OF LIVE PUPS WITH:			
MEAN NUMBER OF BACTERIAL SPECIES	0.503	3.393	0.143
MEAN NUMBER OF SPECIES OF PATHOGENIC BACTERIA	0.211	0.466	0.209
FREQUENCY OF OCCURRENCE OF SPECIES OF PATHOGENIC BACTERIA	0.095	0.510	0.6573

Table 13. Linear regressions of pup weight (kg) and index of condition (kg) with number of bacterial species and number of species of pathogenic bacteria per pup; mean weight and mean index of condition with mean number of bacterial species and mean number of species of pathogenic bacteria per pup, and frequency of occurrence of pathogenic bacteria for rookeries of the Pribilof Islands and Medny Island.

	REGRESSION COEFFICIENT	F VALUE	P VALUE
PRIBILOF AND MEDNY ISLANDS			
WEIGHT OF PUPS WITH:			
NUMBER OF BACTERIAL SPECIES	0.036	0.612	0.435
NUMBER OF PATHOGENIC BACTERIA	0.065	2.003	0.158
PRIBILOF AND MEDNY ISLANDS			
MEAN WEIGHT OF PUPS WITH:			
MEAN NUMBER OF BACTERIAL SPECIES	0.233	0.746	0.430
MEAN NUMBER OF PATHOGENIC BACTERIA	0.544	5.465	0.036
FREQUENCY OF OCCURRENCE OF SPECIES OF PATHOGENIC BACTERIA	0.481	3.915	0.069
PRIBILOF ISLANDS			
CONDITION INDEX WITH:			
NUMBER OF BACTERIAL SPECIES	0.011	4.823	0.029
NUMBER OF PATHOGENIC BACTERIA	0.009	3.958	0.047
MEAN CONDITION INDEX WITH:			
MEAN NUMBER OF BACTERIAL SPECIES	0.009	0.120	0.734
MEAN NUMBER OF PATHOGENIC BACTERIA	0.041	0.554	0.470
FREQUENCY OF OCCURRENCE OF SPECIES OF PATHOGENIC BACTERIA	0.073	1.030	0.329

Figure 1. Location of rookeries on the Pribilof Islands (USA) and Medny Island (USSR).

Figure 2. Linear regression of prevalence (%) of group-D Salmonella and pup mortality (%) for Little Zapadni, Zapadni, Zapadni Reef, Vostochni, and Gorbach rookeries of St. Paul Island.