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Longitudinal Health and Disease Monitoring in Juvenile Steller Sea Lions (*Eumetopias jubatus*) in Temporary Captivity in Alaska Compared with a Free-Ranging Cohort

Tracey Goldstein,[1] Carol A. Stephens,[1] Spencer S. Jang,[2] Patricia A. Conrad,[3] Cara Field,[4] J. Lawrence Dunn,[4] Jo-Ann E. Mellish[1, 5]

[1]Alaska SeaLife Center, 301 Railway Avenue, P.O. Box 1329, Seward, AK 99664, USA
[2]Veterinary Medical Teaching Hospital, Microbiology Laboratory, University of California, Davis, CA 95616, USA
[3]Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616, USA
[4]Mystic Aquarium and Institute for Exploration, 55 Coogan Boulevard, Mystic, CT 06355, USA
[5]University of Alaska Fairbanks, School of Fisheries and Ocean Sciences, Fairbanks, AK 99775, USA
Current Address/Corresponding Author: Tracey Goldstein, The Marine Mammal Center, 1065 Fort Cronkhite, Sausalito, CA 94965, USA; Phone: (415) 289-8609, Fax: (415) 289-7376, E-mail: goldsteint@tmmc.org

Abstract

From March 2003 to June 2006, 77 juvenile Steller sea lions (*Eumetopias jubatus*) from the endangered western stock were captured in Resurrection Bay and Prince William Sound, Alaska. Thirty-one were brought into temporary captivity (transient juveniles) for short-term research studies, and 46 were captured, sampled, and released for a control comparison. The groups of wild-caught sea lions were rotated through a quarantine facility. The objectives of this study were to measure exposure to marine and terrestrial mammalian pathogens in temporarily captive Steller sea lions over time, screen for commensal and pathogenic bacteria, and monitor changes in antimicrobial resistance in bacterial isolates. Antibodies to *Toxoplasma gondii* and *Brucella marinus* were detected in both free-ranging and transient juveniles. Although an increase in titers to *Leptospira* spp. and phocine herpesvirus-1 was detected in a small number of sea lions while housed in temporary captivity, none developed evidence of clinical disease. Additionally, exposure was also found to these potential pathogens in the free-ranging control Steller sea lions. There were no significant differences among the variety of bacterial types obtained from any culture site or animal groups, and antibiotic resistance did not occur in any transient juveniles while in captivity nor in isolates from the free-ranging controls. Results therefore indicated that free-ranging Steller sea lions were not placed at risk for disease following the release of the transient juveniles back into the marine environment.

Key Words: serology, microbiology, temporary captivity, Alaska, Steller sea lion, *Eumetopias jubatus*

Introduction

An increasing number of research-focused facilities are using temporary captivity as a tool to collect vital baseline and experimental data on otherwise difficult to access marine mammal species (Mellish et al., 2006; Greig et al., 2007). One such project was initiated at the Alaska SeaLife Center in Seward, Alaska, in 2003. Following capture, animals were brought into specialized quarantine facilities for short periods for research purposes, after which they were returned to the wild. Risk of exposure to disease for free-ranging populations as a result of such programs is unknown.

Disease transmission is an ongoing concern of institutions that rehabilitate and release injured or compromised marine mammals. Transmission of disease can occur through contact with other individuals at the same facility or through indirect contact with domestic animals while undergoing rehabilitation. Release of these animals is contingent upon their not posing a threat to the wild marine mammal populations from which they came (NOAA, 1972). While disease screening is strongly encouraged, releasability has traditionally been evaluated on the basis of a normal blood panel and lack of significant dependence on human care (St. Aubin et al., 1996). Increased prevalence of diseases such as morbillivirus and *Leptospira* spp. has led to recommendations for the development of more comprehensive disease

screening protocols prior to release (St. Aubin et al., 1996).

Many viral and bacterial diseases have been reported in stranded marine mammals, some of which have been associated with high mortality (Gulland, 1999). Measures (2004) reported concerns that animals admitted for rehabilitation are exposed to diseases and, upon release, carry them to, and therefore may threaten, wild populations. However, stranded harbor seals (*Phoca vitulina*) and California sea lions (*Zalophus californianus*) often have phocine herpesvirus and leptospirosis prior to entry into a rehabilitation center (Gulland et al., 1996; Goldstein et al., 2004). Also of recent concern is the growing evidence of antibiotic resistant bacteria in wildlife (O'Rourke, 2003). Recent work by Stoddard et al. (2005) has shown that bacterial isolates from stranded northern elephant seals (*Mirounga angustirostris*) in rehabilitation compared to those from seals sampled on natal beaches were significantly more likely to be resistant to one or more commonly used antimicrobial drugs. Stranded seals that entered rehabilitation may be more susceptible to infection, which likely accounts for the increase in antimicrobial resistance; however, resistant bacteria were also cultured from seals that had never left their natal rookery, suggesting that these bacteria come from terrestrial sources.

Limited published data are available relative to infectious diseases in Steller sea lions. Evidence of exposure to common marine mammal pathogens, such as phocid herpesviruses, calciviruses, and leptospirosis, has been reported (Calkins & Goodwin, 1988; Burek et al., 2005). Potentially pathogenic bacteria have been cultured upon necropsy from adult females, pups, and fetuses, including *Vibrio fluvialis*, *Edwardsiella tarda*, and *Streptococcus* spp. (Spraker & Bradley, 1996), but, unfortunately, there are few published studies characterizing the normal bacterial flora in this species.

Rotating groups of wild-caught juvenile Steller sea lions (*Eumetopias jubatus*) from the western endangered stock were brought into a specialized facility at the Alaska SeaLife Center for short-term longitudinal research studies. As part of the entry and exit exam protocols, rigorous disease screening was employed. Due to concerns regarding domestic-to-wild species disease transfer, a two-way quarantine was established (Mellish et al., 2006). The monitoring of body condition, general health, and evidence of disease in juvenile Steller sea lions while being held temporarily in captivity was imperative to ascertain whether these sea lions remained clinically healthy throughout their stay in captivity and to ensure that these animals would not place the wild population of Gulf of Alaska Steller sea lions at risk following their release. To accomplish these goals, prevalence of exposure to six common marine and terrestrial pathogens was measured, and a sampling regime was established to screen for normal and pathogenic bacteria as well as to monitor changes in antibiotic resistance over time. Results were compared between free-ranging animals that were captured, sampled, and released and transient juveniles upon capture and exit from captivity.

Materials and Methods

Animals and Samples

From March 2003 to June 2006, 77 free-ranging juvenile Steller sea lions (age 1 to 2 y) were captured at haul-out sites in Resurrection Bay and Prince William Sound, Alaska. Animals were aged using a combination of mass, geographical location (juvenile haulout versus breeding rookery), patterns of tooth eruption (presence of milk teeth), and canine length (King et al., 2007). Thirty-one Steller sea lions were brought to a quarantine facility at the Alaska SeaLife Center for up to 3 mo to be a part of a temporary captive research program (transient juvenile), and 46 were captured, sampled, and released (free-ranging) for a control comparison (Mellish et al., 2006). Animals held in temporary captivity were housed in a separate complex surrounded by a privacy fence that enclosed four adjoining enclosures, each with a fiberglass pool. Adjacent to the pools within the quarantine zone was a dedicated support building that housed food preparation, laboratory, and sampling activities. Unfiltered sea water was pumped directly into the pools, allowing no contact with the water system housing both the captive animals and those undergoing rehabilitation at the Alaska SeaLife Center. To ensure that isolation of the wild animals was maintained, a two-way quarantine shower-in/shower-out protocol was initiated, allowing no contact with domestic, long-term captive, or rehabilitating animals. The captured sea lions were relatively equally distributed by sex: transient juveniles – 12 females and 19 males, free-ranging – 22 females and 25 males. Eight sets of animals were captured over the 3-y period; groups of up to four transient juveniles per cohort were rotated through the facility and the number of free-ranging controls varied from four to ten sea lions in each group.

All animals underwent initial health assessments, including body weight and measurements, complete blood counts, and clinical chemistry analysis. Serum was collected for serologic analysis. Whole blood was collected into sterile serum separation tubes (BD Diagnostics, Franklin Lakes, NJ, USA), allowed to clot for 30 min at

room temperature, then centrifuged. Serum was immediately aliquoted and stored at -80° C until analysis. Nasal, vaginal, and prepuce swabs were collected into Amies transport media without charcoal (BD Diagnostics), and rectal swabs were placed in Cary-Blair transport medium (BD Diagnostics) for bacterial culture. Swabs were held at 4º C until processing. A final comprehensive health assessment repeating sampling for all analyses was performed on all transient juvenile animals at least two weeks prior to release. All sampling was performed while animals were under isoflurane anesthesia to minimize handling and stress.

Serologic Analysis

Samples were submitted to five separate laboratories for serologic analysis. Not all samples were tested for each potential pathogen because of the lack of sufficient serum.

Sera were submitted to Oklahoma Animal Disease Diagnostic Laboratory (OADDL) for testing for antibodies to morbilliviruses (canine distemper, phocine distemper, porpoise morbillivirus, dolphin morbillivirus), phocine herpesvirus-1, and to *Leptospira interrogans* (serovars *pomona*, *hardjo*, *grippotyphosa*, *icterohaemorrhagiae*, *bratislava*). Samples were assayed for exposure to the morbilliviruses by serum neutralization (Saliki & Lehenbauer, 2001; Saliki et al., 2002), and samples were considered to be positive for exposure if titers were greater than 1:16 (twice the cut-off reported by the laboratory). This cut-off was chosen as the assays have not been validated for use in Steller sea lions.

Exposure to phocine herpesvirus-1 also was conducted using a serum neutralization assay which tests for the presence of antibodies against the Atlantic isolate (PB84) of phocine herpesvirus-1 grown in seal kidney cells (seKC) in accordance with methods by Osterhaus et al. (1985). Samples were considered positive if they had a titer of greater than 1:16, again twice the cut-off reported by the laboratory.

A standard microscopic agglutination microtiter (MAT) procedure was used to test for exposure to *Leptospira* spp. (Cole et al., 1979). Titers were determined to be positive for exposure above 1:100 in accordance with methods established for California sea lions (Colagross-Schouten et al., 2002).

Presence of the calicivirus antibody was detected by serum neutralization for exposure to San Miguel sea lion virus (calicivirus serotype 1) at the National Veterinary Diagnostic Laboratory (NVSL) according to previously described methods (Barlough et al., 1986). Samples were all considered negative at a titer of 1:8 or less.

Sera were tested for antibodies to *Toxoplasma gondii* using an indirect fluorescent antibody test (IFAT) validated for use in sea otters (*Enhydra lutris*) (Miller et al., 2002). The IFAT was performed as previously described with the minimum positive cut-off for a sea otter with an active *T. gondii* brain infection of 1:320. The cut-off for exposure (i.e., animals that had developed antibodies but did not have an active infection) was set at 1:160. Since this assay has not been validated for use in Steller sea lions, animals with a titer greater than 1:160 were considered to be positive for exposure to this parasite.

Evidence for exposure to *Brucella* spp. was measured using a competitive enzyme-linked immunosorbent assay (cELISA), which was developed from a whole-cell suspension from a harbor seal *Brucella* isolate, to detect antibodies to marine *Brucella*. All samples were screened at a 1:10 dilution, and the percent of inhibition was measured. Samples with greater than 30% inhibition were considered to be positive for exposure.

Microbiology

All culture and antimicrobial sensitivity testing was performed at the Microbiology Laboratory, Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California at Davis. Swabs were inoculated directly on to trypticase soy agar with 5% sheep blood (Hardy Diagnostics, Santa Maria, CA, USA) and MacConkey agar (Hardy Diagnostics), streaked for isolation, and incubated for 24 to 48 h at 37º C. The blood agar plates were incubated under 5% carbon dioxide and the MacConkey plates were incubated under aerobic conditions. Bacterial isolates were identified and classified according to standardized morphologic and biochemical methods (Nachamkin, 2003; Hirsh et al., 2004; Jang et al., 2005). Undistinguishable isolates were further identified using 16S rRNA gene polymerase chain reaction and sequence analysis, and included gram negative (*Campylobacter* spp., *Enterobacteriacea*, *Stenotrophomonas*, *Pseudomonas* spp.) and gram positive (*Corynebacterium*, non-*enterobacteriacea*) rods and gram positive cocci (*Streptococcal* spp., *Psychrobacter* spp.).

Antimicrobial susceptibility testing was performed on up to four *E. coli* isolates from each rectal swab sample using the broth microdilution method (*Sensititre®*, Trek Diagnostic Systems Inc., Westlake, OH, USA). Inoculation of the minimum inhibitory concentration (MIC) plates was performed as described in the *Sensititre® User Manual*. The MIC plates were incubated at 37˚ C without carbon dioxide overnight and an MIC was determined for each antimicrobial. Antimicrobials tested included amikacin (AMK),

amoxicillin-clavulanic acid (AMC), ampicillin (AMP), cefazolin (CFZ), ceftiofur (CFT), ceftizoxime (ZOX), chloramphenicol (CHL), enrofloxacin (ENR), gentamicin (GEN), tetracycline (TET), ticarcillin-clavulanic acid (TIM), and trimethoprim-sulphamethoxazole (SXT). Methods used for reading and interpreting antimicrobial sensitivity tests were based on Clinical and Laboratory Standards Institute (CLSI) guidelines.

Statistical Analysis

The Fisher exact test (Fisher, 1935) was used to assess differences among groups for prevalence of antibodies when different from zero. The chi-square test and paired *t*-tests were used to compare the number and types of bacteria isolated from each group as well as the means among groups (Zar, 1996). Statistical calculations were performed using *Medcalc*® statistical software, Version 6.0 (1993, Broekstraat 52, 9030 Mariakerke, Belgium) and *SPSS*| (SPSS, Inc., Chicago, IL, USA). A *p*-value < 0.05 was considered statistically significant.

Results

General Health

As reported by Mellish et al. (2006), health was monitored in transient juveniles using body mass and morphometric measurements, complete blood counts, and serum biochemical analysis. Parameters measured upon capture and at exit were compared to parameters obtained from free-ranging juveniles upon capture and immediate release. Overall, temporarily captive animals did not differ from free-ranging juveniles.

Serology

Antibodies to phocine herpesvirus-1, *Leptospira interrogans*, *T. gondii*, and *B. marinus* spp. were detected in both transient and free-ranging juveniles (Table 1). Evidence of exposure to phocine herpesvirus-1 was found in all groups, transient juveniles at capture (9.7%), at exit (19.4%), and free-ranging (2.2%), with titers ranging from 1:24 to 1:96. Three transient juveniles were positive at capture and exit, one from Group 7 and two from Group 8 (two titers declined over the period in captivity from 1:96 to 1:48 and 1:24, respectively; one showed a three-fold increase from 1:24 to 1:96). Although no significant difference was found when comparing exposure to phocine herpesvirus-1 between the transient juveniles and free-ranging animals at capture ($p = 0.30$), the difference was significant between the transient juvenile group at capture and exit ($p = 0.05$), as well as between transient juveniles at exit and the free-ranging group ($p = 0.02$), as three sea lions (one each from Groups 5, 6, and 7) that did not have detectable antibodies at capture did exhibit low titers (1:24 and 1:32) prior to exit.

No antibodies against *L. interrogans* were found in any transient juveniles upon capture; however, low titers (ranging from 1:200 to 1:800) were detected in three transient juveniles from Group 7 at exit (serovars *bratislava* [3.2%] and *hardjo* [9.7%]). Titers to all serovars were found in the free-ranging controls sampled (two individuals

Table 1. Prevalence of antibodies to common mammalian pathogens in transient (sampled at capture and exit) and free-ranging (sampled at capture) juvenile Steller sea lions from 2003 to 2006

Pathogen	Transient juveniles at capture No. positive/no. tested (% positive)	Transient juveniles at exit No. positive/no. tested (% positive)	Free-ranging juveniles at capture No. positive/no. tested (% positive)
Phocine herpesvirus-1	3/31 (9.7%)	6/31 (19.4%)	1/46 (2.2%)
Canine distemper	0/31 (0.0%)	0/31 (0.0%)	0/46 (0.0%)
Phocine distemper	0/31 (0.0%)	0/31 (0.0%)	0/46 (0.0%)
Dolphin morbillivirus	0/31 (0.0%)	0/31 (0.0%)	0/30 (0.0%)
Porpoise morbillivirus	0/31 (0.0%)	0/31 (0.0%)	0/30 (0.0%)
Calicivirus serotype 1	0/31 (0.0%)	0/31 (0.0%)	0/46 (0.0%)
Leptospira canicola	0/31 (0.0%)	0/31 (0.0%)	2/46 (4.3%)
L. grippotyphosa	0/31 (0.0%)	0/31 (0.0%)	2/46 (4.3%)
L. hardjo	0/31 (0.0%)	3/31 (9.7%)	3/46 (6.5%)
L. icterohaemorrhagiae	0/31 (0.0%)	0/31 (0.0%)	3/46 (6.5%)
L. pomona	0/31 (0.0%)	0/31 (0.0%)	3/46 (6.5%)
L. bratislava	0/31 (0.0%)	1/31 (3.2%)	3/46 (6.5%)
Toxoplasma gondii	1/29 (3.4%)	2/25 (8.0%)	1/45 (2.2%)
Brucella marinus	1/29 (3.4%)	1/29 (3.4%)	0/44 (0.0%)

tested positive for all serovars). Low titers (1:160 to 1:320) to *T. gondii* were detected in all groups, transient juveniles at capture (3.4%), at exit (8.0%), and free-ranging (2.2%). One transient juvenile was positive for exposure to *T. gondii* on both entry and exit, with a titer that declined over time. One transient juvenile sea lion also had evidence of exposure to a marine-origin *Brucella* on both capture and exit, while no free-ranging sea lions that were sampled tested positive. No significant differences were found among groups for exposure to any of the latter pathogens. Serologic tests for all four morbilliviruses and calicivirus serotype 1 were negative in all groups. Free-ranging animals that tested positive for exposure to all of the various pathogens were found in almost all of the capture groups, while the transient juveniles that tested positive were from the most recent groups only (Groups 7 & 8).

Microbiology

A total of 642 bacterial isolates were obtained from all of the animals combined, 217 from the transient juveniles at capture, 132 at exit, and 293 from the free-ranging group. These isolates represented 53 genera (Table 2). The majority of isolates were gram negative (n = 476), representing 38 genera, and 16 gram positive genera (n = 179) were cultured. Overall, the most common bacteria isolated were non-hemolytic (114 isolates) and hemolytic (72 isolates) *E. coli*, followed by *Corynebacterium* spp. (66 isolates) and *Psychrobacter phenylpyruvica* (61 isolates).

Thirty-three genera were cultured from the nasal swabs, resulting in 24 isolate types from transient juveniles at capture, 16 on exit, and 21 from the free-ranging group. *Corynebacterium* spp. were most commonly isolated from nasal swabs from all groups (69.8%), followed by *Psychrobacter* spp. (45.8%) and *Serratia* spp. (35.4%). The majority of the *Serratia* isolates were identified as *Serratia marcescens*.

A total of 40 genera were cultured from vaginal swabs and 55 from prepuce swabs. In females, this resulted in 11 isolate types from the transient juveniles on both capture and exit and 18 from the free-ranging sea lions. Eighteen isolate types were cultured from male transient juveniles at capture, 15 on exit, and 23 from the free-ranging group. Overall, the most common genera cultured from vaginal swabs were *E. coli* spp. (47.9%) and *Streptococcal* spp. (35.4%), followed by both *Corynebacterium* spp. and *Psychrobacter* spp. (29.2%). There were differences in the proportion cultured within the groups, however, as *Streptococcal* spp. were most commonly isolated from the transient juveniles at capture, *Psychrobacter* spp. at exit, and *E. coli* spp. from the free-ranging group. Additionally, *Salmonella reading* was isolated from the vaginal swab of one free-ranging individual. Similarly, the same three genera were most commonly cultured from males from prepuce swabs, but in different proportions, as *Psychrobacter* spp. were most common (46%), followed by *E. coli* spp. (38%) and *Streptococcal* spp. (26%).

As would be expected, the most common genera cultured from rectal swabs were *E. coli* (79.6%) and hemolytic *E. coli* spp. (42%) for all groups. Two *Salmonella* spp. were also cultured, serotype *Reading* was isolated from three transient juveniles on entry, and serotype *Newport* from one free-ranging sea lion. Four *Campylobacter* isolates were obtained, two of which were molecularly identified as *Campylobacter insulaenigrae* from one transient juvenile and one free-ranging animal at capture. There were no significant differences among the variety of bacterial types obtained from any culture site or all animal groups. The mean number of isolates from all culture sites combined for transient juveniles at capture was 6.9 (SD = 2.1), 4.3 (SD = 2.4) prior to exit, and 6.6 (SD = 2.8) for the free-ranging individuals. Thus, the number of isolates was not significantly different for all animals at capture ($p = 0.60$); the number cultured did, however, decline significantly prior to exit ($t_{29} = 4.13$, $p < 0.001$). A decrease in the number of isolates cultured from both nasal and rectal swabs accounted for the differences among the groups as significantly more isolates were cultured from both sites at capture compared to exit ($t_{23} = 2.39$, $p = 0.03$ and $t_{26} = 2.11$, $p = 0.04$, respectively). The number of changes in the types of bacteria isolated from the various culture sites in the transient juveniles upon capture and exit ranged from 0 to 7 (mean = 2.9, SD = 0.85).

None of the *E. coli* isolates from the free-ranging animals exhibited any antimicrobial resistance to the 12 antimicrobial drugs tested (Table 3). Three *E. coli* isolates obtained from two transient juveniles upon capture exhibited minor antimicrobial resistance. Two isolates obtained from one animal were resistant to amoxicillin-clavulanic acid, ampicillin, cefazolin, and ceftiofur. The third isolate from a second individual was resistant to gentamicin. These two animals were from Groups 7 and 8. None of the *E. coli* isolates cultured prior to exit from any transient juveniles exhibited antimicrobial resistance.

Discussion

A low prevalence of exposure to phocine herpesvirus-1, *L. interrogans*, *B. marinus*, and *T. gondii* was measured in both transient juveniles and free-ranging sea lions. Phocine herpesvirus has caused high morbidity and mortality in young harbor seal pups in California (Gulland et al., 1997), and

Table 2. Bacterial isolates from the nares, rectum, vagina, and prepuce in juvenile Steller sea lions: transient juveniles at capture (TJ1) and at exit (TJ2) from temporary captivity and free-ranging juveniles (FR) sampled at capture; (*n*) is sample size.

		Number of isolates												
		Nares			Rectum			Vagina			Prepuce			
Group	Bacteria	TJ1 (30)	TJ2 (24)	FR (42)	TJ1 (28)	TJ2 (28)	FR (42)	TJ1 (12)	TJ2 (7)	FR (19)	TJ1 (18)	TJ2 (13)	FR (19)	Total
Gram-positive	*Corynebacterium* spp.	14	13	17	2	--	1	3	3	5	3	3	2	66
	Corynebacterium phocae	7	6	11	--	1	1	2	1	--	--	1	--	30
	Streptococcus viridans	2	2	1	2	--	1	3	1	3	2	2	2	21
	Non-hemolytic *Streptococcus*	--	1	1	2	--	1	4	--	3	1	--	1	14
	Enterococcus spp.	--	--	--	--	2	3	--	--	1	1	--	2	9
	Coagulase-negative *Staphylococcus*	--	--	3	--	--	--	--	--	--	1	--	2	6
	Streptococcus marimammalium	--	--	1	--	--	--	--	--	1	2	--	1	5
	Streptococcus phocae	--	--	--	1	--	--	--	--	--	--	2	--	3
	Beta hemolytic *Streptococcus*	1	--	--	--	--	--	--	--	--	--	1	--	2
	Streptococcus bovis/equinus complex	--	--	--	--	--	--	1	--	1	--	--	--	2
	Atopobacter-like spp.	--	--	--	--	--	--	--	1	--	--	--	1	2
	Bacillus spp.	1	--	--	--	--	--	1	--	--	--	--	--	2
	Arcanobacterium-like spp.	--	--	--	--	--	--	--	1	--	--	--	--	1
	Exiguobacterium spp.	--	--	--	--	--	--	--	--	--	1	--	--	1
	Lactobacillus-like spp.	--	--	--	--	--	--	--	1	--	--	--	--	1
	Micrococcus	--	--	--	--	--	--	--	--	--	--	--	1	1
Gram-negative	*Escherichia coli*	1	--	2	22	19	37	5	2	11	5	2	8	114
	Hemolytic *Escherichia coli*	2	--	--	13	8	21	2	--	12	6	--	8	72
	Psychrobacter phenylpyruvica	8	5	10	3	2	--	7	5	2	8	4	7	61
	Psychrobacter spp.	12	3	12	--	--	1	2	--	3	5	3	5	46
	Serratia marcescens	10	6	17	1	1	--	--	1	--	--	--	--	36
	Non-enteric spp.	6	1	2	--	--	4	--	--	2	2	2	2	21
	Pseudomonas spp.	2	--	5	--	--	1	--	1	2	--	2	4	17
	Actinobacillis/Bisgaardia spp.	1	1	3	--	--	--	--	1	--	4	2	1	13
	Stenotrophomonas spp.	2	--	4	--	--	--	--	--	1	2	--	3	12
	Pseudomonas flourescent	1	1	1				1	--	--	4	1	--	9
	Ornithobacterium spp.	2	--	3	--	--	--	--	--	--	--	--	2	7
	Enterobacter spp.	--	--	3	--	--	--	--	--	--	--	--	3	6
	Paracoccus spp.	2	2	--	--	--	--	--	--	1	--	--	1	6

Bisgaardia hudsonenesis	--	1	--	--	--	--	--	--	--	1	3	--	5
Brevundimonas/Caulobacter-like spp.	1	--	3	--	--	--	--	--	1	--	--	--	5
Aeromonas spp.	1	--	--	--	--	1	--	--	--	2	--	--	4
Edwardsiella tarda	--	--	--	1	1	1	--	--	--	--	--	1	4
Kingella/Cardiobacterium spp.	--	4	--	--	--	--	--	--	--	--	--	--	4
Moraxella-like spp.	2	1	1	--	--	--	--	--	--	--	--	--	4
Pleisomonas shigelloides	--	--	--	--	--	2	--	--	1	--	--	--	3
Pseudomonas aeruginosa	1	1	--	1	--	--	--	--	--	--	--	--	3
Salmonella newport	--	--	--	3	--	--	--	--	--	--	--	--	3
Campylobacter spp.	--	--	--	--	--	2	--	--	--	--	--	--	2
Campylobacter insulaenigrae	--	--	--	1	--	1	--	--	--	--	--	--	2
Morganella morganii	--	1	1	--	--	--	--	--	--	--	--	--	2
Pantoea spp.	--	--	2	--	--	--	--	--	--	--	--	--	2
Salmonella reading	--	--	--	--	--	1	--	--	1	--	--	--	2
Stenotrophomonas maltophilia	--	--	--	--	1	--	--	--	--	--	--	1	2
Buttiauxella gaviniae	--	--	--	--	--	--	--	--	--	1	--	--	1
Chryseomonas spp.	--	--	--	--	--	--	--	--	--	--	1	--	1
Flavimonas-like spp.	1	--	--	--	--	--	--	--	--	--	--	--	1
Neisseria spp.	--	--	--	--	--	--	--	--	--	--	--	1	1
Providencia spp.	--	--	--	--	--	--	--	--	1	--	--	--	1
Psychrobacter faecalis/pulmonas	--	--	--	1	--	--	--	--	--	--	--	--	1
Psychrobacter glacincola	--	--	--	--	--	--	--	--	--	--	1	--	1
Rahnella aquatilis	1	--	--	--	--	--	--	--	--	--	--	--	1
Serratia spp.	1	--	--	--	--	--	--	--	--	--	--	--	1
Total													642

Table 3. Mean minimum inhibitory concentration (MIC) values[a] and standard deviation (SD) for *E. coli* isolated from transient juvenile Steller sea lions (sampled at capture and at exit) and free-ranging juvenile Steller sea lions and tested for resistance to 12 commonly used antimicrobials

Group	*n*	Number *E. coli* isolates	Resistant *E. coli* (%)	Antimicrobial mean MIC (SD)											
				AMK	AMX	AMP	CFZ	CFT	ZOX	CHL	ENR	GEN	TET	TIM	SXT
TJ Capture	27	67	4.5%	1.64 (1.25)	2.96[b] (2.47)	2.45[b] (2.51)	2.46[b] (2.41)	0.33[b] (1.06)	0.66 (1.01)	4.72 (1.96)	0.25 (0.00)	0.7[b] (1.91)	1.21 (0.57)	8.00 (0.00)	0.25 (0.00)
TJ Exit	27	48	0.0%	1.34 (0.66)	2.46 (0.85)	1.96 (0.74)	2.08 (0.40)	0.16 (0.08)	0.50 (0.00)	3.92 (0.82)	0.26 (0.05)	0.45 (0.19)	1.24 (0.48)	8.00 (0)	0.25 (0)
Free-ranging	42	113	0.0%	1.37 (0.71)	2.73 (0.97)	2.03 (0.82)	2.00 (0.00)	0.17 (0.08)	0.50 (0.00)	4.16 (1.26)	0.25 (0.00)	0.48 (0.17)	1.08 (0.32)	8.00 (0.00)	0.25 (0.00)

[a]Mean MIC values are all susceptible based on Clinical Laboratory Standards Institute (CLSI) guidelines
AMK = amikacin, AMX = amoxicillin-clavulanic acid, AMP = ampicillin, CFZ = cefazolin, CFT = ceftiofur, ZOX = ceftizoxime, CHL = chloramphenicol, ENR = enrofloxacin, GEN = gentamicin, TET = tetracycline, TIM = ticarcillin-clavulanic acid, SXT = trimethoprim-sulphamethoxazole
[b]Antimicrobials showing resistance to three bacterial isolates

evidence of exposure to likely a closely related virus has been previously measured by Burek et al. (2005). That study, however, reported a slightly higher prevalence of exposure to phocine herpesvirus-1 of 30% in animals of the same age class compared to this study. Although the antibody titers were low, transient juveniles prior to exit from temporary captivity in this study had a significantly higher level of exposure to this pathogen than when they entered captivity and compared to the free-ranging group. Herpesviruses are known to reactivate during times of stress (Roizman, 1982), and so it is possible that the stress of handling and isolation in captivity resulted in recrudescence of a latent virus in at least one animal that could have resulted in a new infection in others, therefore accounting for the increase in antibody titers. Alternatively, the titers could have been false positives as the assay was developed for detection of exposure to phocine herpesvirus-1 in harbor seals. Nonetheless, since antibodies to phocine herpesvirus-1 were found both in free-ranging Steller sea lions in this study and by Burek et al. (2005), release of these juveniles from temporary captivity with either a new or reactivated infection would not pose a risk of exposure to a novel disease to the free-ranging Steller sea lion population.

Leptospirosis is a common infection of California sea lions and northern fur seals (*Callorhinus ursinus*), both of which overlap with the range of the Steller sea lion (Smith et al., 1977; Gulland et al., 1996). Negative or very low titers have been previously reported in Steller sea lions to *Leptospira* spp. (1:100 to serovars *icterohaemorrhagiae* and *grippotyphosa* and 1:200 to serovar *bratislava*) (Calkins & Goodwin, 1988; Burek et al., 2005). While the overall prevalence was also low in animals in this study, positive titers were higher than those previously reported and ranged from 1:200 to 1:1,600. Evidence of exposure was measured to all six serovars in two individuals from the free-ranging group, with one animal having a titer of 1:1,600 to *L. interrogans* serovar *pomona*. These titers to *Leptospira* spp. were below those measured in California sea lions considered to be positive for this infection (Colagross-Schouten et al., 2002), indicating that a low level of exposure to this potential pathogen may have occurred but currently does not appear to be a significant disease in Steller sea lions. Three transient juveniles from Group 7 had antibodies to *L. interrogans* serovar *hardjo* prior to exit that were not measured upon entry. It is possible that these were false positives or that at least one of these animals was a chronic carrier of the infection and began to shed the bacteria in urine while at the facility, thus exposing the other two sea lions to infection.

Antibodies to *T. gondii* have not been previously measured in Steller sea lions. Evidence of exposure to *T. gondii*, a protozoal parasite that causes severe encephalitis in sea otters and harbor seals (Miller et al., 2001, 2002), has been found in other pinnipeds in Alaska (Dubey et al., 2003). Three animals (two transients and one free-ranging) had titers of 1:160 and 1:320 to *T. gondii*. Since the titers reported here were low and the test has not been validated for use in Steller sea lions, it is unlikely that these animals had an active protozoal infection. Similarly, this is the first time that exposure to marine *Brucella* spp. has been measured in Steller sea lions, although testing for *B. abortus* has been performed. *Brucella* spp. have not been previously isolated from Steller sea lions, but it is important to monitor for this pathogen because it is zoonotic, causes chronic infections, and can affect reproduction in wildlife

and other species (Godfroid, 2002). Only one transient juvenile had antibodies to *Brucella* at both capture and exit. It is unclear how to interpret this result, but likely exposure to *Brucella* is not a concern in these animals.

Caliciviruses are common marine viruses, and a relatively high prevalence of exposure has been found in many pinniped and cetacean species (Smith et al., 1998). Surprisingly, no animals tested positive for San Miguel sea lion virus, whereas 20% of juvenile Steller sea lions previously tested were positive (Burek et al., 2005). This difference could be due to our inability to test for exposure to strains other than serotype 1, while previously testing for serotypes 5, 6, 10, and 13 was possible. It was not surprising that antibodies to the marine mammal morbilliviruses were not measured in the animals in this study as they have not been isolated from a marine mammal in the Pacific (Duignan, 1999). Nonetheless, it is important to continue surveillance for exposure to these viruses because mass mortality associated with an infection could have devastating consequences for the susceptible marine mammal populations in Alaska.

A variety of bacteria were cultured from swabs from the animals in this study. Overall, *E. coli* spp. were the most common bacteria isolated. This finding is consistent with other studies in which *E. coli* was also the most frequently bacteria cultured from other pinnipeds (Thornton et al., 1998; Lockwood et al., 2006). It is important to note, however, that those studies were reporting bacteria cultured from inflammatory lesions, whereas the cultures in this study were from clinically healthy animals. Also, frequently cultured in this study was *Psychrobacter phenylpyruvica*. This bacterium has been reported as an inhabitant of the marine ecosystem (Bowman et al., 1996); therefore, it is not surprising to commonly culture it from these animals. Additionally, *P. phenylpyruvica* was the most frequent isolate from free-ranging California sea lions on San Miguel Island off California (Johnson et al., 2006).

Corynebacterium spp., *Serratia* spp., and *Psychrobacter* spp. most often were isolated from nasal swabs. Both of the former species have been cultured from the nares of phocid seals (Pascual et al., 1998; Lockwood et al., 2006). *S. marcescens* is thought to be an opportunistic pathogen in livestock and humans and interestingly has become an important bacteria in nocosomial infections in recent years (Hejazi & Falkiner, 1997). Similarly, *E. coli* spp., *Streptococcal* spp., *Corynebacterium* spp., and *Psychrobacter* spp. were most often cultured from vaginal and preputial swabs and have been reported to be common isolates from these sites in clinically healthy, free-living California sea lions (Johnson et al., 2006). As would be expected, *E. coli* and hemolytic *E. coli* spp., both of which are typical flora of the gastrointestinal tract of mammals, were the most common bacteria cultured from rectal swabs. Overall, few potential pathogens were cultured from all sites in these animals, some of which included *Streptococcus phocae*, *Campylobacter insulaenigrae*, *Plesiomonas shigelloides*, and *Salmonella reading* and *S. newport*. The role of *S. phocae* in morbidity and mortality in pinnipeds is unknown, but the bacterium was associated with pneumonia in harbor seals dying with morbillivirus (Kuiken et al., 2006). The other bacteria have been associated with diarrheal disease in many species and are potential zoonoses (Namchamkin, 2003), so care should be taken when handling these animals. Since all of these animals were clinically healthy, however, the significance of these isolates is unknown. Reports of other potentially pathogenic bacteria cultured from free-ranging Steller sea lions in the mid-1990s included *Edwardsiella tarda*, *Listeria*, *Salmonella*, *E. coli*, and *Streptococcus* spp. (Spraker & Bradley, 1996), suggesting that healthy Steller sea lions may carry opportunistic pathogens that may cause bacterial infections if the animals become stressed and or immuno-suppressed. The bacteria in this study were also all isolated from clinically healthy animals, and as a result, may represent transient and or commensal organisms in Steller sea lions.

While many bacteria were isolated from all animals, there was no difference between either the number or types of bacteria cultured from transient and free-ranging juveniles upon capture. Furthermore, the number of bacteria isolated from transient juveniles prior to release decreased significantly. While there were some differences in the bacteria cultured from individual animals at capture and exit from temporary captivity, overall, the types of bacteria cultured from each site in each group were relatively similar. Results therefore indicate that animals did not increase their bacterial loads while in captivity.

Few antimicrobial-resistant *E. coli* isolates were cultured. Those that were cultured were only from transient juveniles upon capture and prior to entry to the facility, and no isolates from these animals showed any antimicrobial resistance prior to exit. Development of antimicrobial resistance is enhanced by the use of antimicrobial drugs and disinfectants (Russel et al., 1998). These juveniles were healthy and not treated with antimicrobials while in captivity; however, they were exposed to disinfectants such as bleach, chlorhexidine, and Trifectant™ (Vetoquinol USA/Evsco Pharmaceuticals, Buena, NJ, USA), indicating that monitoring of antibiotic resistance was prudent. Recent work has shown that elephant seal

pups undergoing rehabilitation had an increase in prevalence of resistant *E. coli* isolates to multiple antimicrobials compared to free-ranging seals regardless of antimicrobial usage (Stoddard et al., in review). Fortunately, this did not occur in this study. Resistance to broad spectrum antimicrobials, such as the β-lactam antibiotics, is common in enterobacteria (Georgopapadakou, 2002) and was seen in two transient juveniles upon capture. The potential sources of these resistant bacteria in these Alaskan pinnipeds is unknown but may include fresh water run-off and sewage outfall carrying waste from humans and domestic animals as well as waste from other wildlife, all of which have been previously documented (Sayah et al., 2005). Additionally, a high prevalence of antimicrobial resistant isolates of *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Helicobacter pylori* associated with infections and disease have been found in rural Alaskan natives (Parkinson et al., 2000; Rudolph et al., 2000; Baggett et al., 2003). None of these bacteria were cultured from these animals; however, data suggest that multiple drug resistance could occur in Alaskan wildlife and patterns should continue to be monitored in animals brought into temporary captivity.

Overall, results showed that Steller sea lions held in temporary captivity for up to 3 mo remained healthy. While several potentially pathogenic bacteria were identified, no evidence of disease in Steller sea lions was associated with any of these organisms. Additionally, the minimal antibiotic resistance measured was present upon capture and prior to housing in captivity. Although an increase in titers to *Leptospira* spp. and phocine herpesvirus was found in a small number of transient juveniles upon exit exam, none of them developed any evidence of clinical disease, and antibodies were found to the same potential pathogens in the free-ranging control Steller sea lions. Since this increase was measured in transient juveniles from the last two groups and two animals from these groups also harbored antibiotic-resistant bacteria upon capture, continued surveillance is important to ensure that a new pattern of increased infection within the facility is not developing. In conclusion, our results indicate that free-ranging Steller sea lions were not placed at risk for new disease following the release of transient juveniles back into their marine environment. It is nonetheless important to continue to monitor the health and to update protocols as needed to include surveillance for new diseases as they emerge.

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