Primary Infectious Disease Concerns in Pinnipeds of Washington for 2004

***Dyanna M. Lambourn (lambodml@dfw.wa.gov)[1], Steven .J. Jeffries[1], Stephan A. Raverty[2], Michael M. Garner[3], Stephanie A. Norman[4], Joseph K. Gaydos[5], John Calambodikidis[6], Melissa E. Miller[7], Patricia A. Conrad[7] and Caroline E. Cameron[8]**

[1]*Washington Department of Fish and Wildlife, Marine Mammal Investigations, 7801 Phillips Road S.W., Lakewood, WA 98498* [2]*Animal Health Center, B.C. Ministry of Agriculture, Food and Fisheries, Canada,* [3] *Northwest ZooPath,,* [4]*NOAA Fisheries, Protected Resources Division,* [5]*UC Davis Wildlife Health Center-Orcas Island Office,* [6]*Cascadia Research Collective,* [7]*Wildlife Heath Center, School of Veterinary Medicine, UCD and* [8]*Department of Medicine, University of Washington,*

Abstract

The Washington Department of Fish and Wildlife, Marine Mammal Investigations unit (WDFW/MMI) is one of the primary responders in the Northwest Marine Mammal Stranding Network and has been responding to stranded marine mammals for over 20 years. In the past, stranding response and thorough case workups have been limited due to time, funding constraints, and other research priorities. In 2004 WDFW/MMI received funding from a NOAA Fisheries Prescott Marine Mammal Stranding Grant to investigate and monitor causes of marine mammal mortality in Washington waters. For the time period specified in the grant awarded from December 1, 2003 to November 30, 2004, 274 calls were recorded with 166 field numbers assigned and 75 necropsies conducted. Tissue specimens were systematically harvested and submitted for histopathology and ancillary diagnostic workups on all code 2 and some code 3 carcasses. There were 17 live stranded animals. Those animals that were recovered alive and either died shortly thereafter, or were euthanized, are included in the necropsy total. Based on results obtained from post mortem examinations during for 2004, primary established and emerging disease concerns in stranded harbor seals (*Phoca vitulina)* included *Brucella* spp infections and protozoal encephalitis due to *Sarcocystis neurona*, respectively. Incursion of leptospirosis into Oregon and Washington associated with northward migrating male California sea lions (California – *Zalophus californianus* and Steller – *Eumetopias jubatus*) was documented between August and December 2004.

Introduction

John H. Prescott Marine Mammal Rescue Assistance Grant Program was established due to the enactment of the Marine Mammal Rescue Assistance Act of 2000 (MMPA Title V, section 408). Washington Department of Fish and Wildlife, Marine Mammal Investigation (WDFW, MMI) applied for and was awarded funds to respond to stranded marine mammals and investigate causes of mortality in Washington waters starting December 1, 2003.

WDFW/MMI is a primary response organization participating in the Northwest Marine Mammal Stranding Network and is listed in the National Contingency Plan for Marine Mammal Stranding Response.

WDFW/MMI works close cooperation with other Northwest Stranding Network Participants to include NWR NOAA fisheries, Cascadia Research Collective, Central Puget Sound Marine Mammal Stranding Network, SeaDoc Society, Whale Museum, PAWS Wildlife, Wolf Hollow Rehabilitation Center and numerous other local animal control agencies and State Park authorities.

Objectives

•Respond to reports of stranded marine mammals- primarily southern Puget Sound and Hood Canal. Expanded to include all of the western Washington and northern Oregon coastline, due to protozoal encephalitis (due to *Sarcocystis neurona*) in harbor seals and outbreak of leptospirosis in northward migrating California sea lions.

•Conduct thorough necropsies of stranded marine mammals to assess causes of mortality (e.g. human interactions, disease or other natural causes).

•Screen for pathogens of concern, including Morbillivirus, *Toxoplasma gondii* , *Sarcocystis neurona*, *Neospora caninum*, select serovars of *Leptospira interogans* and *Brucella* spp all of which may potentially contribute to ill health and increase mortality in marine mammal stocks.

Methods

Call Response

•Marine mammals strandings are responded to during the week, weekend and holidays.
•Staff assists in covering NWR stranding hotline on weekends and holiday.
•Assists in dispatching and coordinating responses by appropriate stranding network participants.
•Call log maintained with all stranding calls received, assigns field numbers to animals responded to by WDFW staff
•Log is transmitted weekly to NMFS Northwest Region and Level A data is entered online.

Live Stranding Response

•Developed live stranding protocol for pinnipeds to determine action level required in response to stranding calls.
•Animals handled, are triaged and blood is drawn for diagnostics and disease screening.
•Released animals are tagged and complete necropsies are performed on animals that are euthanized.

Dead Stranding Response

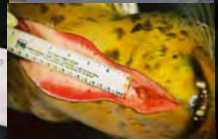
•Postmortem examination is performed to determine cause of death on on all code 2 and some code 3 carcasses.
•Tissue systematically harvested, portions are put in formalin and individual whirlpaks, jars, labeled and frozen.
•Samples submitted for histopathology, ancillary diagnostic workups, *Brucella* culture and contaminants
•Laboratories included: AHC, NWZP, AFIP, PCL, UCD, WDA, NVSL, PC

Disease Screening

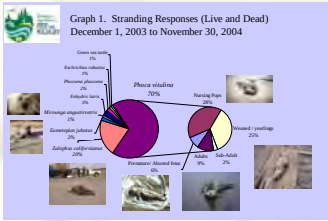
•Serum samples are routinely submitted to WDA screen for *Brucella* spp and *Leptospira* spp.
•Sample may also be submitted to screen for other diseases depending on necropsy finding and availability of serum.
•Frozen tissue samples were archived, submitted to the NVSL for special *Brucella* spp culture and identification
• If *Brucella* spp is isolated by culture, ancillary studies, may be pursued.

WDFW Stranding Call Log

Date	Time	Caller Name	Contact #	Location	Species	Animal status	Comments	Actions taken	Call location	WDFW Log #
01-Jan-04	13:00	Suquamish Dispatch	(360) 340-4526	Just N. of Kingston Ferry at the beach access	P.v.	Alive	Kingston parking partol reported it to Kitsap county sheriff > contacted Suquamish dispatch >Chris Lane, WDFW oil spill	Responded immediately, arrived on site, contacted Kitsap Sheriff told that seal was put back in water by citizen and swam off	WDFW	0104-
02-Jan-04	12:30	Mark Frankfurt	(206) 431-0609	S. of The Cove boat ramp, Normond y Park	P.v.	Dead	Br 427. Flesh missing from skull hole in back. ~ 4 ft long	Responded, and necrops		
				Bremerton Passenge				NMFS (Step she was goi		



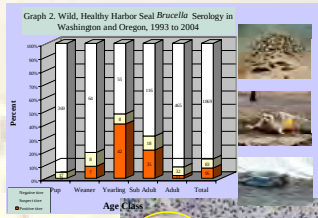
Results



Graph 1. Stranding Responses (Live and Dead) December 1, 2003 to November 30, 2004

Call Log and Stranding Response

- A total of 274 marine mammal stranding calls were received
- 166 animals assigned a WDFW/MMI Field Number.
- A total of 75 post mortem examinations performed
- 17 live stranded animals handled (Graph 1).



Graph 2. Wild, Healthy Harbor Seal *Brucella* Serology in Washington and Oregon, 1993 to 2004

Harbor Seal Tissues that *Brucella* spp. was Cultured and Number of Colonies.

Tissues Sampled	n=	Percentage of culture positives (%)	Number of colonies grown
Lymph nodes			
Sublingual, Submandibular, Supra scapular, Supra scapular Inguinal, Mesenteric, Mediastinal, Pulmonary	44	75	10 - >1000
Uterus, Broad, Spleen, Gastric, Reproductive, Hepatic, Cardiac	30	67	1-50
Organs			
Lung	7	43	10 - Too Growth
Testes	4	50	1-999
Kidney	7	14	10-99
Reproductive tract	7	14	10-99
Pancreas	7	14	10-99
Uterus	3	29	1-10
Urinary Bladder	3	50	1-10
Brain	1	25	>10
Eye	2	33	>10
Gall Bladder	2	50	>10
Liver	1	28	>10
Salivary gland	3	33	> 10
Synovial tissue	1	33	>10
Thymus	1	100	> 10
Tonsil	4	75	> 10

*The first Brucella spp. culture from a harbor seal in the Pacific Northwest was in 1996 and recently Brucella has been isolated or confirmed by Polymerase Chain Reaction (with uniserra Brucella spp primers) on 20 harbour seals from Washington State.

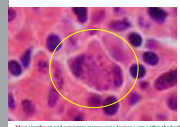
Primary Infectious Disease Concerns:

Marine Mammal *Brucella*

- Initially documented in harbor seals and other marine mammals by serology in the Pacific Northwest in 1993
- Ongoing serosurveys on healthy and stranded marine mammals in Washington and Oregon
- Since 1993, 14% of all wild healthy harbor seals had positive/suspect titres (Graph 2)
- Evidence significantly higher among yearlings (48%) and sub adults (31%)
- Currently *Brucella* has been isolated or confirmed by PCR on twenty harbor seals from Washington State (Table 1 tissues cultured).
- IHC disclosed coccobacilli within the uteri of *Parafilaroides* lungworms
- From an epidemiologic perspective, seropositive/culture positive harbor seals were most frequently identified from November through to February and comprised predominantly young of year and sub adults
- At present, there is insufficient data to speculate on what may account for these age-class related differences.

Positive IHC staining in lung and coccobacilli within the uteri of Parafilaroides septungorum

Clinical signs: Reluctant to return to water, head , spasm or convulsion, frothing from mouth, raised, inflamed third eyelids and approachable by humans.



Most significant and consistent microscopic lesions were within the brain and comprised of a nonsuppurative and occasionally necrotizing meningitis to meningoencephalitis with varying numbers of protozoal cysts.

Harbor Seal Protozoan Encephalitis

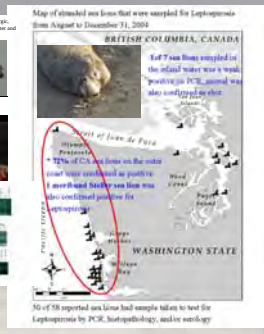
- May -July, 2004, along Washington to mid Oregon coast
- 10 reports of sub adult to adult harbor seals stranded and moribund
- 5 were collected and necropsied
- Additionally, 3 pups (2 in July, S Puget Sound,and 1 in September, San Juan)
- **Diagnosis: Protozoal encephalitis due to *Sarcocystis neurona* confirmed on serology, IHC and PCR**
- Negative for *Toxoplasma gondii* and *Neospora spp.*
- Negative morbillivirus (canine distemper virus) IHC and PCR
- No domoic acid was detected within the ingesta of all outer coast animals.

California Sea Lion Leptospirosis

- WDFW/MMI coordinated collection and tracking sea lions to assess impact of leptospirosis in WA
- 58 moribund and dead stranded sea lions in Washington State waters August - December 2004
- Serologic, molecular and histopathology studies confirmed leptospirosis, due to *Leptospira intergans var Pomona*
- 1 moribund sub-adult male Steller sea lion (1/1)
- 72% (13/18) of California sea lion sampled from the outer coast
- 1 weak positive in 7 California sea lions sample within the inland water Puget Sound

Clinical sign: Very poor nutritional condition, lethargic, weak, reluctant to use hind limbs, drinking fresh water and were occasionally uremic

Gross exam: Disclosed enlarged kidneys due to fulminant interstitial nephrits



Marine Mammal Morbillivirus

- Potential for explosive epizootics has prompted widespread and ongoing surveillance for PDV and CDV
- 157 live healthy harbor seals were screened from Oregon and Washington from 1989 to 1996
- 48 harbor seals, 13 California sea lions in 2004 were negative on PCR/ IHC for morbillivirus
- To date- No positives harbor seals or California Sea lions reported in Washington.
- 1 case of distemper (due to CDV) was confirmed in a dead sea otter in 2004.

Conclusion

Improved diagnostic evaluation and understanding of disease processes in stranded and live captured pinnipeds in Washington State was afforded by the Prescott Grant funds and continued support will expanded efforts to further understand natural diseases processes and potential anthropogenic impacts on in marine mammals within the Pacific Northwest. Through the 2004 NOAA Fisheries Prescott Marine Mammal Stranding Grant, WDFW/MMI has increased its ability to respond to stranded marine mammals in western Washington and with continued funding plans to enhance the level of stranding response for 2005 and the future.

Acknowledgements

NOAA Fisheries John H. Prescott Marine Mammal Rescue Assistance Grant Program and Washington Department of Fish and Wildlife, Cascadia Research Collective have provided funding and support for these research activities. Thanks to all the agencies and individuals that are part of the Northwest Marine Mammal Stranding Network. Especially, all the WDFW enforcement officers, biologists and scientific technicians that assist in stranding response outside there normal duties. WDFW/MMI stranding volunteers especially Tammy Schmidt, Carolyn Rosevelt, Kelly Brock, Kim Laub and Mike Walker. NWR of NOAA Fisheries, Protected Resource Division and Marine Mammal Laboratory, especially Brent Norberg, Lynne Barre, Harriet Huber, Pat Gearin and John Scordino. Cascadia Research Collective especially Jessie Higgins and Dominque Comancho. Island County Stranding Network especially Sue and Mike Murphy, Susan Berta and Sandy Duprenell and the staff and volunteers at PAWS Wildlife and Wolf Hollow Rehabilitation Centers.