

***Vibrio parahaemolyticus*, a Climate Change Indicator in Alaska Marine Mammals**

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Abstract

Since 1999, the Alaska SeaLife Center has routinely screened live marine animals found in distress, and those found dead, for fecal pathogens (e.g., *Salmonella*, *E. coli* 0157, *Campylobacter*, *Vibrio* spp.) and exposure to a variety of diseases known to affect marine mammals and/or humans (e.g., *Brucella*, *Morbillivirus*, *Leptospirosis*, *Herpesvirus*). Additionally, projects investigating wild populations screen live cap-

tured animals and subsistence harvested animals for the same conditions circulating in wild populations. One fecal pathogen in particular, *Vibrio parahaemolyticus* (*Vp*), is considered an indicator of climate change as this organism proliferates only in waters with temperatures higher than 15°C. The State of Alaska began screening oysters for *Vp* in 1995, but did not detect *Vp* until summer 2004 when the first human outbreak of *Vp*-associated gastroenteritis was documented, involving 62 people who consumed raw oysters from Prince William Sound. Since then, marine mammal researchers on stranding and live capture projects have found *Vp*, including isolates with known pathogenicity factors, in several (17 stranded, 2 live captured, 1 subsistence) northern sea otters (*Enhydra lutris kenyoni*), a harbor porpoise (*Phocoena phocoena*), and a beluga whale (*Delphinapterus leucas*). The positive *Vp* isolates found in Seward, Cook Inlet, Kachemak Bay, Kodiak, and Dillingham represent the first reports of the bacteria in those areas.

Introduction

Marine animals found live in distress or dead are examined as part of Alaska stranding programs across the state. Live and dead animals that are processed through the Alaska SeaLife Center (ASLC) come primarily from Prince William Sound, Cook Inlet, and Bristol Bay. These animals are routinely screened for fecal pathogens (e.g., *Salmonella*, *Escherichia coli* 0157, *Campylobacter*, *Vibrio* spp.) and exposure to a variety of diseases known to affect marine mammals and/or humans (e.g., *Brucella*, *Morbillivirus*, *Leptospirosis*, *Herpesvirus*). The Alaska Veterinary Pathology Service (AVPS) and the US Fish and Wildlife Service (USFWS) do similar testing as part of ongoing projects investigating causes of mortality of marine mammals from across the state. Additionally, projects investigating wild populations often screen live captured animals and subsistence-harvested animals for these same conditions. At lower latitudes, episodic and seasonal warming are associated with increased levels of fecal pathogens and human illness. At higher latitudes the fecal pathogen *Vibrio parahaemolyticus* (*Vp*) is considered to be an indicator of climate change because it proliferates only in waters with sustained temperatures higher than 15°C, which are unusual in the north (Rose et al. 2001, McLaughlin et al. 2005, Broberg et al. 2011, Baker-Austin et al. 2012, Nelapati et al. 2012).

Illness from *Vp* is most commonly associated with the ingestion of raw seafood, primarily filter-feeding mollusks, and causes gastroenteritis in people. The disease is characterized by vomiting and diarrhea; it does not typically require treatment with antibiotics and resolves in a few days with only supportive therapy such as rehydration with fluids. However, *Vp* can cause death in individuals who are immunocompromised by concurrent diseases or use of immunosup-

pressive drugs (Daniels et al. 2000, Broberg et al. 2011). Additionally, *Vp* can cause localized wound infections associated with environmental exposure (Rose et al. 2001, Broberg et al. 2011, Baker-Austin et al. 2012). Until recently, the northernmost environmental *Vp* isolate was found in shrimp waste and wastewater from a seafood processing site in Petersburg, Alaska (detected in 1973). This isolate was serotype 08:K39, Kanagawa negative, but was not typed for genes associated with virulence (Vasconcelos et al. 1975). From 1995 to 2003, the Alaska Department of Environmental Conservation (DEC) tested oysters for *Vibrio* species but did not detect any *Vp* (McLaughlin et al. 2005). During that time the northernmost report of disease in humans was in British Columbia, Canada, detected in 1997 (CDC 1998). Historical thinking was that the cold water temperatures found in the North Pacific Ocean would prevent *Vp* from existing or proliferating to levels that would cause disease in Alaska. However, climate change is now altering physical and biological ocean characteristics, which in turn is altering the distribution and pathogenicity of waterborne bacteria (McLaughlin et al. 2005, Baker-Austin et al. 2012, Parkinson and Butler 2012). Increased sea surface temperatures and decreasing salinity of coastal waters due to increased freshwater input from increased rainfall, glacial melting, and terrestrial runoff, ultimately optimize environmental conditions for *Vp*, which proliferates to infectious levels at temperatures above 15°C and salinities less than 25 ppt (DePaola et al. 2000, Baker-Austin et al. 2012). Additionally, plankton provides a substrate or reservoir for *Vibrio* species that can then be amplified, along with their plankton hosts, to infectious levels in the event of a bloom (Rose et al. 2001, Nelapati et al. 2012).

Water temperature affects not only the distribution of *Vp* but also the presence of pathogenicity factors such as thermostable direct hemolysin (encoded by the gene *tdh*) in *Vp* strains (McLaughlin et al. 2005, Mahoney et al. 2010, Broberg et al. 2011, Ellis et al. 2012, Nelapati et al. 2012). Such was suspected in summer 2004 when Alaska had its first outbreak of *Vp*-associated gastroenteritis affecting 62 cruise ship passengers who consumed raw oysters from Prince William Sound (McLaughlin et al. 2005). Subsequent analysis of environmental isolates associated with that outbreak demonstrated that very low levels of bacteria (3.5 colony forming units per gram of shellfish, lower than the US Food and Drug Administration level of concern at 10,000 colony forming units per gram) (DePaola et al. 2000) were able to cause disease. In addition, 74% were *tdh*-positive; the highest previously documented environmental concentration found anywhere was 3% *tdh*-positive (McLaughlin et al. 2005). While *Vp* may not be culturable or infectious at temperatures below 15°C it is now recognized that it can be present and viable (Nelapati et al. 2012). The pattern of virulence in the 2004 Alaska outbreak suggests that *tdh*-positive, more virulent strains

might be associated with cold tolerance. If so, this could result in an increased percentage of the bacterial population being pathogenic should water temperatures reach 15°C and significant proliferation occurs (McLaughlin et al. 2005).

Since 2004, investigations of marine mammals in Alaska, including clinical examinations of stranded animals and health assessments of live captured wild animals, have detected *Vp*, including isolates with virulence factors in opportunistically collected samples.

Materials and methods

Case information for this report was obtained from a retrospective review of records from multiple projects in which animals were screened for fecal pathogens. Sampled animals included stranded marine mammals found dead or live animals in distress, animals captured as part of other projects, and subsistence harvested animals made available for biosampling. Details on the individual projects are listed below and summarized in Table 1.

As part of its stranding program the Alaska SeaLife Center (ASLC) examined and screened 229 animals for fecal pathogens including 140 harbor seals (*Phoca vitulina*), 16 ringed seals (*Phoca hispida*), 7 spotted seals (*Phoca largha*), 40 Steller sea lions (*Eumetopias jubatus*), 15 harbor porpoise (*Phocoena phocoena*), and 11 beluga whales (*Delphinapterus leucas*) during 1999-2012. In addition to participating in the study of dead sea otters described here, ASLC admitted and screened 18 live stranded sea otters that were subsequently transferred or released.

As part of a project to understand causes of mortality and morbidity of northern sea otters in Alaska, USFWS, AVPS, and ASLC examined and screened for fecal pathogens 182 animals from southeastern to southwestern Alaska from 1999 to 2013.

During field captures to instrument northern sea otters (*Enhydra lutris kenyoni*), USFWS also obtained biosamples and screened 113 sea otters for fecal pathogens (39 from Kodiak in August 2005, 43 from Kachemak Bay in August 2007, and 31 from southeastern Alaska in May 2011).

While admitting juvenile Steller sea lions captured from Prince William Sound to the ASLC for short-term physiology and nutrition studies, 62 animals were examined and screened for fecal pathogens during 2003-2010.

During field captures to instrument beluga whales in Bristol Bay, National Marine Mammal Laboratory (NMML), ASLC, and AVPS obtained biosamples and screened 19 beluga whales for fecal pathogens (9 in May 2008, 8 in September 2008, and 2 in September 2012).

Rectal swabs, feces, and other biological samples were obtained and screened for infectious agents (see below) using microbial culture

at Providence Alaska Medical Center or University of California Davis Veterinary Medical Teaching Hospital Microbiology Laboratory. Samples were cultured for common enteric pathogens, including *Salmonella*, *Shigella*, *Campylobacter jejuni*, *Escherichia coli* O157, and *Vibrio* spp.

While not possible for every project, a subset of positive *Vp* isolates was confirmed by culture at the Alaska State Public Health Laboratory (ASPHL, Anchorage, AK) by plating to Trypticase Soy Agar with 5% Sheep Blood (BD BBL, Franklin Lakes, NJ) at 35°C for 24 hours and identified using the Vitek 32 (bioMérieux, Cambridge, MA). Further characterization of the subset of positive *Vp* isolates was performed using Pulsed Field Gel Electrophoresis (PFGE). Macrorestriction of DNA from the positive *Vp* isolates was performed using restriction enzymes *SfiI* and *NotI* (New England Biolabs, Ipswich, MA) according to the *Vp* PulseNet protocol (Parsons et al. 2007). DNA macrorestriction using the restriction enzyme *ApaI* (Roche, Indianapolis, IN) was performed according to the same protocol with slight modifications to the switch times (2.2 s to 45 s) to increase discrimination. Cluster analysis was performed using BioNumerics (Version 5.1, Applied Maths, Austin, TX) and the dice coefficient using the unweighted pair group method with arithmetic averages (UPGMA). PFGE of the isolates from the 2004 Alaska *Vp* outbreak was previously performed by the Centers for Disease Control and Prevention (CDC, Atlanta, GA) (McLaughlin et al. 2005). Slide agglutination serotyping and PCR for genes encoding for virulence factors was performed at the CDC. Isolates were serotyped by slide agglutination using antisera to 11 *Vibrio parahaemolyticus* O antigens and 71 K antigens (Denka Seiken, Co., LTD., Tokyo, Japan), tested for urease and oxidase production, and tested by PCR for genes encoding the thermostable direct hemolysin (*tdh*), thermolabile hemolysin (*tlh*), and the thermostable direct-related hemolysin (*trh*).

Results

Vibrio parahaemolyticus was found in northern sea otters (2 of 113 live captured animals, 17 of 182 dead stranded animals, 1 subsistence hunted animal, and 0 of 18 live stranded animals), a harbor porpoise (1 of 15), and a beluga whale (1 of 19 live captured animals, 0 of 11 dead stranded animals) (Table 1). Culture positive animals came from Homer, Seward, Cordova, Cook Inlet, Kachemak Bay, Kodiak, and Dillingham. Numerous Steller sea lions (40 stranded, 62 live captured) and stranded phocid seals (140 harbor seals, 16 ringed seals, and 7 spotted seals), were negative for *Vp*. While more than half of the isolates from animals were found during the summer (7 in July, 4 in August, 3 in September), *Vp* was found throughout the year in Alaska, including January.

Two *Vp* isolates obtained from sea otters in 2009 were confirmed by culture at the ASPHL. Further characterization of these two isolates

Table 1. Summary information for projects screening for *Vibrio parahaemolyticus* (*Vp*) and significant events documenting *Vp* in the North Pacific Ocean. Events involving *Vp* are highlighted in gray. (NOTE: All locations are Alaska except one in British Columbia. PWS = Prince William Sound, CI = Cook Inlet, SE = Southeast Alaska, SW = Southwest Alaska, COD = cause of death, ASLC = Alaska SeaLife Center, AVPS = Alaska Veterinary Pathology Service, FWS = US Fish and Wildlife Service, NMML = National Marine Mammal Laboratory.)

Species	Date	Location	Comments, findings (data source)
Environmental samples	1973	Petersburg	Seafood processing investigation (Vasconcelos et al. 1975)
Human outbreak	1997	British Columbia, Canada	51 human cases traced to oysters harvested on BC coast (CDC 1998)
Harbor seal	1999-2012	PWS, CI, SE	0 of 140 positive for <i>Vp</i> , Strandings, various causes (ASLC)
Spotted seal	1999-2011	Bristol Bay	0 of 7 positive for <i>Vp</i> , Stranding, various causes (ASLC)
Ringed seal	1999-2011	North Slope	0 of 16 positive for <i>Vp</i> , Stranding, various causes (ASLC)
Steller sea lion	1999-2012	PWS, SE	0 of 40 positive for <i>Vp</i> , Stranding, various causes (ASLC)
Steller sea lion	2003-2010	PWS	0 of 62 positive for <i>Vp</i> , Captured for physiology studies (J. Mellish, ASLC, Seward, AK unpubl. data)
Harbor porpoise	2005-2011	PWS, CI	1 of 15 positive for <i>Vp</i> , Stranding, various causes (ASLC), Positive case detailed below (2005)
Beluga whale	2007-2012	CI	0 of 11 positive for <i>Vp</i> , Stranding, various causes (ASLC)
Northern sea otter	1998-2012	Rangewide (SE, PWS, CI, SW)	0 of 18 positive for <i>Vp</i> , Live stranding, various causes (ASLC)
Northern sea otter	1999-2013	Rangewide (SE, PWS, CI, SW)	18 of 182 positive for <i>Vp</i> , Positive cases detailed below, Stranding, various causes (USFWS, AVPS, ASLC)
Human outbreak	Jul-04	PWS	62 human cases traced to oysters harvested from oyster farms in PWS (McLaughlin et al. 2005)
Northern sea otter	Jun-05	Homer	Stranding, COD trauma (ASLC, AVPS, FWS)
Northern sea otter	Jul-05	Homer	Stranding, COD cerebellar atrophy (ASLC, AVPS, FWS)
Northern sea otter	Jul-05	Seward	Stranding, COD trauma (ASLC, AVPS, FWS)
Northern sea otter	Jul-05	Cordova	Stranding, COD pneumonia (ASLC, AVPS, FWS)
Harbor porpoise	Jul-05	Cook Inlet	Stranding, COD entanglement (ASLC, AVPS, FWS)
Northern sea otter	Aug-05	Kodiak	1 of 39 positive for <i>Vp</i> , Captured for tagging (FWS)

Table 1. Continued

Species	Date	Location	Comments, findings (data source)
Northern sea otter	Aug-07	Kachemak Bay	1 of 43 positive for <i>Vp</i> , Captured for tagging (FWS)
Northern sea otter	Sep-07	Kachemak Bay	Stranding, COD other infection (ASLC, AVPS, FWS)
Northern sea otter	Mar-08	Kachemak Bay	Subsistence-harvest, (Healthy) (AVPS, FWS)
Beluga whale	May-08	Dillingham	1 of 9 positive for <i>Vp</i> , Captures (1 of 9) (ASLC, AVPS, NMML)
Beluga whale	Sep-08	Dillingham	0 of 8 positive for <i>Vp</i> , Captures (0 of 8) (ASLC, AVPS, NMML)
Northern sea otter	Aug-09	Cook Inlet	Stranding, COD cerebral abscess (ASLC, AVPS, FWS)
Northern sea otter	Aug-09	Kodiak	Stranding, COD neoplasia (ASLC, AVPS, FWS)
Northern sea otter	Nov-09	Kodiak	Stranding, COD trauma (AVPS, FWS)
Northern sea otter	Sep-10	Cordova	Stranding, COD undetermined (AVPS, FWS)
Northern sea otter	Sep-10	Kachemak Bay	Stranding COD undetermined (AVPS, FWS)
Northern sea otter	Mar-11	Seward	Stranding, COD emaciation (AVPS, FWS)
Northern sea otter	May-11	SE	0 of 31 positive for <i>Vp</i> , Captured for tagging (FWS)
Northern sea otter	Jan-12	Cordova	Stranding, COD emaciation (AVPS, FWS)
Northern sea otter	Apr-12	Cordova	Stranding, COD emaciation (AVPS, FWS)
Beluga whale	Sep-12	Dillingham	0 of 2 positive for <i>Vp</i> , Captured for tagging and health study (ASLC, AVPS, NMML)
Northern sea otter	May-13	Homer	Stranding, COD pyothorax (AVPS, FWS)
Northern sea otter	Jul-13	Homer	Stranding, COD enterocolitis (AVPS, FWS)
Northern sea otter	Jul-13	Homer	Stranding, COD pulmonary edema (AVPS, FWS)
Northern sea otter	Jul-13	Homer	Stranding, COD heart failure (AVPS, FWS)

using pulsed field gel electrophoresis (PFGE) indicated that the isolates were unique, different from each other and from the isolates found during the 2004 human outbreak. Serotyping identified the otter isolates as O2:Kunknown and O:Kunknown whereas the isolate from the 2004 human outbreak was previously identified by the CDC as serotype O6:K18. Additionally, PCR analysis to determine the presence of specific genes demonstrated that both of the 2009 otter isolates were positive for the species-specific thermolabile hemolysin (*tlh*), and one was positive for virulence factor thermostable direct hemolysin (*tdh*).

Discussion

Increasing water temperature and decreased salinity of coastal areas, events associated with global climate change, are important factors in the proliferation of many bacterially mediated waterborne diseases, contributing to a worldwide increase in disease events and the emergence of these diseases in new, more northerly areas (Baker Austin et al. 2010, Parkinson and Butler 2012). Until this report, the only *Vp* of Alaska origin was documented in Petersburg (Vasconcelos et al. 1975) and Prince William Sound (McLaughlin et al. 2005). The geographic distribution of the *Vp* isolates reported here demonstrates the presence of *Vp* in previously undocumented areas, including Seward, Cook Inlet, Kachemak Bay, Kodiak, and Dillingham.

The majority of the isolates reported here were cultured from animals found or captured in summer. However, some isolates were identified in winter months, suggesting either persistence in the environment or a carrier state in the animals in which it was found. The majority of animals that were culture positive for *Vp* were northern sea otters, which may be in part due to greater numbers of otters being tested for fecal pathogens. Additionally, northern sea otters may have greater exposure to *Vp* as many of the favored prey items of sea otters include filter feeders that can concentrate bacteria (Miller et al. 2006, 2010; Riedman and Estes 1990), whereas the diet of Steller sea lions and harbor seals has a greater proportion of fish (Hoover-Miller 1994, Waite et al. 2012).

The *Vp* infections reported here were incidental findings, not a cause of significant disease or death in the animals in which it was found. All of the *Vp* positive animals that were captured alive and the one subsistence-harvested animal were considered healthy. Additionally, *Vp* was found in a healthy captive otter. A second test of that individual was negative, suggesting that the animal cleared the bacteria from its digestive tract without the aid of antibiotics, or that the first result was a false positive (C. Goertz, ASLC, Seward, AK, unpubl. data). A few sea otters with additional, more serious health concerns did have some pathology attributed to *Vp*, namely enteritis and septicemia,

but the interpretation was that these changes occurred because the animal was sufficiently debilitated by its primary problem and cause of death (Table 1). While these infections did not affect the health of the marine mammals, these cases are a public health concern as the sea otters likely acquired *Vp* from local prey that are also consumed by people. In addition to commercial shellfish operations in Prince William Sound, recreational and subsistence shellfish harvesting takes place across the state including Cook Inlet (Alaska Administrative Code, 5 AAC 77.507) where many of the *Vp* positive sea otters were collected.

Alterations in commercial oyster farming practices can reduce the bacterial load sufficiently to reduce the risk of human disease. These techniques include lowering oysters to temperatures below 15°C for 24 hours prior to harvest and other depuration methods in which oysters are held in clean, sterilized water and allowed to excrete most of the bacteria from their digestive tracts. However, this is not routinely done unless the presence of *Vp* is first detected or at least suspected. Monitoring programs are sporadic, especially in Alaska, and tend to focus on commercial operations and often leave areas with subsistence and recreational harvests untested. In place of bacterial monitoring programs, information about the local sea surface temperature can be used to assess the relative risk. Additionally, information and isolates from *Vp* culture positive marine mammals can be shared with public health officials for further testing and analysis in order to be able to alert the public. Finally, individual harvesters can further reduce their risk of acquiring *Vp* by chilling live shellfish in cold circulating water, below 15°C, for 24 hours, refrigerating harvested shellfish as quickly as possible, and thoroughly cooking shellfish.

Finding *Vp* in marine mammals underscores the importance of these animals as sentinels for diseases circulating in the oceans. This testing is useful for understanding the distribution of a pathogen that affects human health, especially in areas that are not routinely tested but are used in subsistence fisheries. Also, finding *Vp* indicates that the waters from which the animals came maintained temperatures above 15°C for a sufficient amount of time to allow *Vp* to proliferate to infectious levels. Finally, the positive *Vp* isolates found in Seward, Cook Inlet, Kachemak Bay, Kodiak, and Dillingham represent the first report of the bacteria in those areas.

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