Western Wildlife 6:69–81 • 2019
Submitted: 20 September 2019; Accepted: 10 December 2019.

Survey for *Coccidioides* Antibodies in Blood Sera from California Sea Lions and Northern Fur Seals that Stranded Along the Coast of California (2013–2015)

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***Abstract.*—Coccidioidomycosis, also known as Valley Fever, infects marine mammals along the coast of California. These animals reside far from the Central Valley of California and the Mojave Desert where the fungal pathogen *Coccidioides immitis* is endemic. An agar gel immunodiffusion (AGID) assay established to screen for coccidioidomycosis exposure in humans and canines was used on blood sera collected from 136 stranded California Sea Lions (*Zalophus californianus*) of all age groups and 17 Northern Fur Seals (*Callorhinus ursinus*) between 2013 and 2015, to quantify the prevalence of exposure to the pathogen. We detected *Coccidioides*-specific antibodies in approximately 11% of California Sea Lions and 24% of Northern Fur Seal pups that were diagnosed with respiratory problems at time of admission to two marine mammal care centers. No significant difference was detected in risk of exposure to the pathogen when comparing sex, or stranding location of California Sea Lions, however, we found that yearlings of California Sea Lions did not show any evidence of seroconversion. Our results confirm that exposure to *Coccidioides* occurs among pinnipeds, likely due to strong Santa Ana winds, which carry dust from mainland California to the Pacific Ocean. Thus, disease resulting from infection with *Coccidioides* should be considered as a possible cause for stranding in pinnipeds. The risk of exposure to the pathogen can be considered equal for California Sea Lions, Northern Fur Seals, and other marine mammals that spend significant time in an area affected by mainland dust. The location of greatest exposure is difficult to determine, however, because of sea lion migration behavior and variable wind and weather patterns. The findings of this study have implications for marine mammal rehabilitation and conservation, and also for public health.**

Key Words.—agar gel immunodiffusion assays; *Callorhinus ursinus*; coccidioidomycosis; immunoglobulin M; immunoglobulin G; marine mammal rehabilitation; pinnipeds; stranding; *Zalophus californianus*

Introduction

Major causes of pinniped (seals and sea lions) strandings along the coast of California include natural or manmade disasters, storm surges, El Niño events (that often result in malnutrition), human or predator related injuries, infectious diseases, and non-infectious diseases (cancer, congenital, etc.) including domoic acid poisoning (Gerber et al. 1993; Colegrove et al. 2005; Greig et al. 2005). Malnutrition lowers the functional performance of the immune system and renders animals more vulnerable to infectious diseases (Simeone et al. 2015). Stranded pinnipeds that suffer from malnutrition are often also diagnosed with pneumonia, which can be caused by various infectious agents (viruses, bacteria, fungi, and/or parasites). The cause of pneumonia, and other infectious diseases, must be identified for successful treatment, rehabilitation, and release of a rescued marine mammal.

In coastal California, pinnipeds and other marine mammals (i.e., Sea Otters [*Enhydra lutris*], dolphins, and whales) have occasionally been diagnosed with coccidioidomycosis, also known as Valley Fever, a disease caused by a soil-borne fungal pathogen (Reed et al. 1976; Cornell et al. 1979; Fauquier et al, 1996; Kirkland and Fierer 1996; Reidarson et al. 1998; Carlson-Bremmer et al. 2012). Recently, coccidioidomycosis was identified as the most common mycosis in stranded marine mammals along the central California coast (Huckabone et al. 2015; Simeone et al. 2015). Unfortunately, the disease is often diagnosed postmortem, when treatment with antibacterial drugs was unsuccessful or when supportive care of animals failed. At that stage, the disease likely disseminated throughout the body, and affected lymph nodes, lungs, and bones (Gerber et al. 1993; Shubitz and Dial, 2005; Simpson and Cornell 2018). For example, over a six-year period, 22.2% of rehabilitated pinnipeds in California presented with pneumonia of unknown origin (suspected bacterial or verminous infections). Some of these animals might have contracted coccidioidomycosis (Gerber et al. 1993) but were not diagnosed. Between 2005 and 2014, 12 California Sea Lions (*Zalophus*

californianus) rescued at The Marine Mammal Center (TMMC) succumbed to coccidioidomycosis (Appendix Table). Early detection and proper treatment of coccidioidomycosis could have improved the outcomes for these stranded animals. Understanding the potential sources of this pathogen, typically associated with dry, desert habitats, may be helpful to determine when and where testing for the pathogen may be a cost-effective diagnostic tool for marine mammal rescue efforts.

The opportunistic fungal pathogens, *Coccidioides immitis* and *C. posadasii*, both cause coccidioidomycosis. In California, *C. immitis* is highly endemic in the central and southern San Joaquin Valley and the western Mojave Desert where it can be found in non-disturbed fine particulate soils (alluvium) dominated by salt bushes (particularly Allscale, *Atriplex polycarpa,* Spiny Saltbush, *A. spinifera*, Shadscale, *A. confertifolia*, and Fourwing Saltbush *A. canescens*) and Creosote (*Larrea tridentata*), whereas *C. posadasii* is established in other areas of the Southwestern U.S., Mexico, and parts of South America (Baptista-Rosas et al. 2007; Fisher et al. 2007). Arthroconidia (dormant forms of the pathogens) can become airborne when soil is disturbed and when inhaled, arthroconidia can form spherules in the lung, potentially resulting in pneumonia and occasionally disseminating to other internal organs (Pappagianis 1980).

The migratory behavior of marine mammals hinders precise determination of where these animals may be exposed to the pathogen. California Sea Lions tend to migrate long distances along the U.S. west coast and can range from southern Mexico and Baja California, where *C. posadasii* resides, to British Columbia (considered non-endemic for *Coccidioides*). Rookeries (breeding grounds) are located almost entirely on the Channel Islands of California, western Baja California, and the Gulf of California (Aurioles et al. 1983; Laake et al. 2018). Therefore, it can be inferred that pinnipeds spend significant time in waters and rookeries exposed to mainland dust potentially containing *C. immitis* (California) or *C. posadasii* (Mexico) and are likely at risk of exposure while in these areas. Northern Fur Seals (*Callorhinus ursinus*), which have established rookeries on San Miguel Island close to the *C. immitis* endemic mainland, are at risk of pathogen exposure as well, but instead of migrating south, these pelagic animals prefer the north Pacific Ocean with another large rookery on the Farallon islands near San Francisco, California; however, most breeding grounds of this species are in the Aleutians Islands in the Bering Sea.

There are two likely sources of the pathogen: fugitive dust from offshore islands and from areas known to be endemic for *C. immitis* and/or *C. posadasii* on the mainland. Although many of the Channel Island plant communities resemble comparable communities on the California mainland, they are dominated by endemic species unique to the islands. In response to soil and climate conditions that are different from the mainland, communities on the islands form distinct plant alliances and associations such as Island Chaparral, Coastal Sage Scrub, and Grassland. *Atriplex polycarpa* and other *Atriplex* species, as well as Creosote that are adapted to saline-alkaline desert soils, can indicate a suitable habitat for *Coccidioides*, but are not established on the Channel Islands (Junak et al. 2007; Channel Island National Park. 2019. Channel Islands Plant Check List. National Park Service, U.S. Department of the Interior. Available from https://www.nps.gov/chis/planyourvisit/upload/A-Checklist-of-Vascular-Plants-all-v1.pdf [Accessed 6 October 2019]). Therefore, it seems unlikely that *Coccidioides* are an important part of the fungal community in Channel Islands soils.

The recent surge (from about 2010 to now) in land development in the California deserts for utility-scale solar energy development (USSED), in addition to fallow farmland resulting from a long-term drought, has contributed to an increase in fugitive dust and coccidioidomycosis in highly endemic areas of the pathogen in the Mojave Desert (Hector et al. 2011; Thompson III et al. 2015; Guevara et al. 2015; Grayzel et al. 2017). Recent studies in the western Mojave Desert, located north of the San Gabriel Mountains that separates the Los Angeles Basin from the Mojave Desert, revealed the presence of *C. immitis* in 29% of all soil samples collected from disturbed and non-disturbed soils at sites destined for large-scale renewable energy projects west of Lancaster, California (Etyemezian et al. 2018). Since 2011, a significant increase in coccidioidomycosis among humans has been observed in Kern (southern San Joaquin Valley) and northern Los Angeles (Mojave Desert) counties (Cooksey et al. 2017; Colson et al. 2017). Recent research also revealed an earlier onset of the spring fine dust season in the endemic area of the pathogen due to climate change (Hand et al. 2016), which likely leads to an increased risk of exposure to *Coccidioides* for animals and humans.

Strong seasonal Santa Ana winds arriving at the end of the long summer drought season (May-September/October) in California can transport pathogenic arthroconidia from dry and eroded land in the Mojave Desert to the Los Angeles Basin and beyond, putting people as well as domestic and wild animals at risk of developing coccidioidomycosis (Westerling et al. 2004; Duniway et al. 2019; Appendix Figure). Between 2013 and 2017, strong Santa Ana winds carried dust from the Mojave Desert to non-endemic areas of the pathogen along the coast and fanned several wildfires (Kolden and Abatzoglou 2018). These events increased particulate matter concentrations of 10 μm or less in diameter (PM10) in the air along the coast of California and are a growing public health issue (Black et al. 2017).

In anticipation of regular Valley Fever surveillance in the future, we aimed to obtain initial information about how common exposure to *Coccidioides* is among stranded

California Sea Lions admitted to two marine mammal care centers in California over a 3-y period. A small number of Northern Fur Seal pups was also included in this study because of unusually high numbers of strandings in 2014 and 2015. Here we present preliminary data from a series of planned tests that will continue monitoring coccidioidomycosis in California Sea Lions and Northern Fur Seals through 2021 (NOAA permit # 19706). Valley Fever monitoring would also be of interest to scientists at the National Oceanic and Atmospheric Administration (NOAA) Fisheries agency (https://www.fisheries.noaa.gov/national/marine-life-distress/2013-2017-california-sea-lion-unusual-mortality-event-california) who are investigating California Sea Lion Unusual Mortality Events (CSL UME).

Methods

Study sites.—Pinnipeds admitted to the Marine Mammal Care Center Los Angeles (MMCCLA) in San Pedro, California, and to The Marine Mammal Care Center (TMMC) in Sausalito, California, were included in this study. The MMCCLA admits animals stranded in Los Angeles County (Malibu to Seal Beach); whereas, TMMC responds to animals stranded between San Luis Obispo County and Mendocino County. Both Marine Mammal Care centers (collectively, MMCs) identify the causes of stranding, document and diagnose stranded animals, and treat them with the intent of rehabilitation and release.

Blood serum samples.—We tested stored, frozen blood serum samples from California Sea Lions and Northern Fur Seals rescued between 2013 and 2015 for antibodies against *Coccidioides* spp. Additional information was gathered as available, including the cause of stranding, any diagnoses and analyses performed by the veterinarians at the MMCs at any stage of treatment at the site of recovery, and whether the animal was released. Information gathered from animals found dead or that were euthanized while in rehabilitation (e.g., lesions in the lung, in bones, or in inner organs) was included as well. We focused on stranded California Sea Lions and Northern Fur Seals diagnosed with a respiratory disease (suspected pneumonia) because respiratory problems can indicate coccidioidomycosis in an early stage (Pappagianis 1980; Valdivia et al. 2006). Overall, we analyzed serum samples from 136 California Sea Lions (all age groups; MMCCLA San Pedro: n = 70, TMMC Sausalito: n = 66). In addition, we included 17 stranded Northern Fur Seals (16 pups and one subadult) that stranded in 2014 and 2015 in this study.

Immunodiffusion assays.—We performed an agar gel immunodiffusion (AGID) assay for *Coccidioides* specific immunoglobulin M (IgM) and immunoglobulin G (IgG) on serum samples. Samples were frozen, aliquots were prepared by personnel of the MMCs, and were shipped on dry ice to the Public Health Laboratory at Kern Medical in Bakersfield, California. These assays were performed following the method described in Pappagianis and Zimmer (1990). This method is identical to the method performed at the School of Veterinary Science of the University of California, Davis (UC Davis), which is used to diagnose coccidioidomycosis in humans, dogs, and occasionally marine mammals (Pappagianis 2001; Gautam et al. 2013; Schmitt and Procter 2014).

Statistical analyses.—We limited all statistical tests to California Sea Lions because the number of positive AGID assays from Northern Fur Seal pups was too small to conduct meaningful statistical analyses (n = 17, with four positive tests). Furthermore, we pooled all positive immunodiffusion assays from California Sea Lions because a positive test for either IgG or IgM indicates exposure to the pathogen. We conducted statistical analyses based on the binary variable of exposure (positive vs. negative) for either test for each animal.

We used the Log-Likelihood Ratio test for Contingency Tables (Zar 1999; McDonald 2014) to determine if AGID test results of blood serum from California Sea Lions were significantly different between individual years from 2013–2015, sexes, and age classes. We conducted post-hoc power analyses with Gpower, assuming either a moderate effect size of 0.3 or a small effect size of 0.1. Finally, we used the G-test (Woolf 1957) with Yates correction for continuity (Haviland 1990) to analyze if stranding location (by county) might be indicative of a higher risk of *Coccidioides* exposure in California Sea Lions.

Results

We screened 136 serum samples from California Sea Lions admitted to MMCCLA or TMMC between 2013 and 2015 for the presence of *Coccidioides* specific antibodies IgM and IgG using AGID assays to investigate the prevalence of exposure to the pathogen. Serum from adult female sea lions comprised most samples obtained from animals rescued by MMCCLA at San Pedro (about 77%, n = 54); whereas, samples from TMMC in Sausalito had a fairly even distribution of sex and age groups. For both MMCCs combined, about 36% of the sea lions were adults and about 16% were pups (Table 1).

We tested California Sea Lions of all age groups that stranded between Los Angeles County and Mendocino County. Overall, 51% of serum samples were from animals recovered by MMCCLA in San Pedro and 49% from animals rescued by TMMC Sausalito (Table 2). Positive immunodiffusion assays for IgM ranged from about 8–14% between 2013–2015. The prevalence of positive IgG test results was low and ranged between about 2–3% in each year (Table 3). The proportions of California Sea Lions testing positive for IgM or IgG

Table 1. Number of stranded California Sea Lions (*Zalophus californianus*), by sex and age class, admitted to Marine Mammal Care Center Los Angeles in San Pedro (MMCCLA), California, and to The Marine Mammal Center in Sausalito (TMMC), California, between 2013 and 2015 that presented with a respiratory infection (n = 136) and which contributed to this study. Distribution % is the distribution of age classes from all animals at both Marine Mammal Care Centers.

	MMCCLA (n = 70)	TMMC (n = 66)	Distribution % (n = 136)
Females	54	34	64.71
Males	16	32	35.29
Pup	2	20	16.18
Yearling	2	18	14.71
Juvenile	0	6	4.41
Subadult	23	16	28.68
Adult	43	6	36.03

across years was not significantly different (G = 1.427, P = 0.499). There was also no significant difference in risk of exposure to the pathogen between sexes (with year and age groups pooled; G = 0.007, P = 0.935); however, we observed a significant difference between age groups and exposure to the pathogen (with years and sex pooled; G = 10.12, P = 0.018; Table 4).

Stranding in an area closer to the endemic regions of the pathogen (Los Angeles Basin) was also not an indicator of increased risk of exposure to the pathogen in these cases (Yates corrected G = 0.020, df = 1, P = 0.888). In Los Angeles County about 13% of the 70 stranded sea lions tested positive for either IgG (1.43%) or IgM (11.43%), compared to 10.6% (3.03% positive for IgG, 7.57% positive for IgM) for all other counties combined (n = 66; Fig. 1). Overall, 10.86% of animals that stranded in either area had detectable amounts of IgM or IgG.

Table 2. Percentage of California Sea Lions (*Zalophus californianus*) of all age classes (n = 136) and Northern Fur Seals (*Callorhinus ursinus*), with few individuals, mostly pups (n = 17), that were part of this study, sorted by stranding location (counties, from south to north). The sea lion from Stanislaus County (not a coastal county) lived in a zoo.

Counties	California sea lions % (n = 136)	Northern fur seals % (n = 17)
Los Angeles	51.47	11.77
Santa Barbara	8.82	0
San Luis Obispo	8.08	29.40
Monterey	11.03	5.88
Santa Cruz	5.88	11.77
Stanislaus	0.74	0
San Mateo	7.35	5.88
Alamedo	0.74	0
San Francisco	2.21	5.88
Marin	1.47	5.88
Sonoma	2.21	11.77
Mendocino	0	11.77
Total	100%	100%

Results of the AGID assays from blood sera of Northern Fur Seals identified four of 17 animals positive for *Coccidioides* exposure. Two animals were positive for *Coccidioides* specific IgM only, while two additional fur seals were positive for both antibodies (all pups). No fur seals were positive for IgG alone. Most of these animals stranded in San Luis Obispo County, but strandings occurred as far north as Sonoma and Marin counties. The two Northern Fur Seal pups rescued in Los Angeles County were negative for both antibodies. Overall, 24% of Northern Fur Seal pups were positive: 12% of the pups tested positive for IgM alone and 12% tested positive for both IgM and IgG. Of the four animals that showed an immune response to *Coccidioides* antigens, two stranded in 2014 and two in 2015.

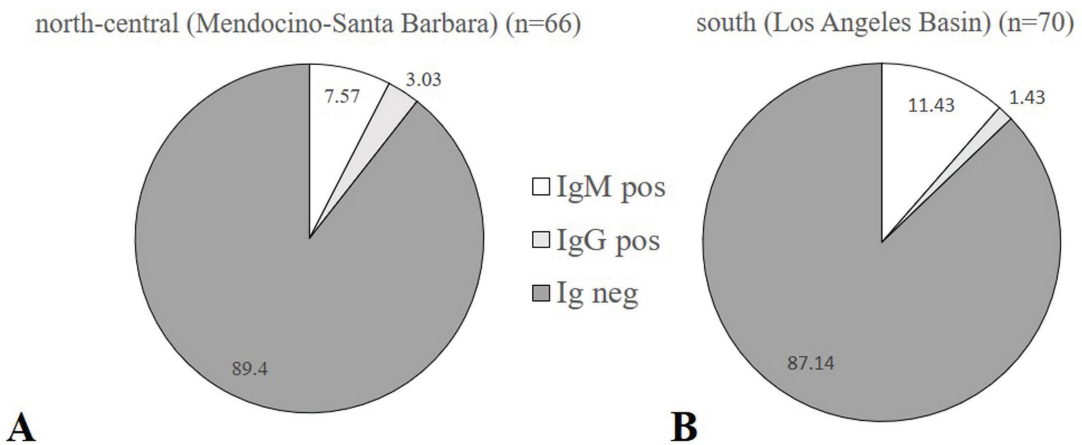

Figure 1. Results of agarose gel immunodiffusion assays for *Coccidioides* specific antibodies (IgM and IgG) comparing the percentage of California Sea Lions (*Zalophus californianus*) that (A) stranded in north-central counties (Mendocino-Santa Barbara County) of California and (B) the percentage of animals that stranded in Los Angeles County.

Table 3. Results of agarose gel immunodiffusion assays for blood serum from 136 California Sea Lions (*Zalophus californianus*) that stranded between 2013 and 2015 and which were diagnosed with respiratory problems including suspected pneumonia. Results are separated by year of stranding and by age classes. The abbreviation n = sample size.

		Sex		Positive AGID assays	
Age classes	n	Females	Males	IgM	IgG
2013					
Adult	22	21	1	3	0
Subadult/Juvenile	8	6	2	1	1
Yearling	1	0	1	0	0
Pup	5	4	1	1	0
Total n	36	31	5	5	1
Percentage	100	86.11	13.89	13.89	2.78
2014					
Adult	21	17	4	1	0
Subadult/Juvenile	16	7	9	2	0
Yearling	5	4	1	0	0
Pup	9	4	5	2	1
Total n	51	32	19	5	1
Percentage	100	61.54	37.25	9.8	1.92
2015					
Adult	7	7	0	0	1
Subadult/Juvenile	20	8	12	3	0
Yearling	13	13	0	0	0
Pup	8	3	5	0	0
Total n	49	24	25	3	1
Percentage	100	48.98	51.02	8.33	2.78
Total n (2013–2015)	136	87	49	13	4

Discussion

Our results support earlier studies showing that California Sea Lions and Northern Fur Seals living along the coast of California or those migrating through this area are at risk of exposure to *C. immitis*, a fungal pathogen that may be transported to coastal areas via strong Santa Ana winds from endemic areas in the Mojave Desert (Simeone et al. 2015; Huckabone et al. 2015). This fugitive dust can be transported to the South Coast Air Basin that includes areas in Los Angeles, Orange, Riverside, and San Bernardino counties. Extreme weather events have been associated with cases of coccidioidomycosis in traditionally non-endemic areas of the pathogen, including one in a gorilla in the Sacramento Zoo (Pappagianis and Einstein 1978). The Northridge earthquake in 1994 is another event that transported dust and *Coccidioides* arthroconidia north to Sacramento, California, and into Oregon (Schneider et al. 1997). We cannot exclude the possibility that *C. immitis* persists or grows in soils near pinniped rookeries, however, possibly inoculated by transported dust. Muhs (1983) and Muhs et al. (2007, 2008) provided evidence from soil and dust minerology studies supported by Landsat imagery that aeolian dust originating from the Mojave Desert deposits regularly on the Channel Islands where pinniped rookeries are established (Laake et al. 2018). Muhs (1983) estimates that dust deposit rates on San Clemente Island range between 28–31 g/m$^{2/y}$, reflecting ongoing and accelerated erosion of soil surfaces disturbed by human activity in the Mojave Desert, which has increased in recent years (Sprigg et al. 2014; Tong et al. 2017). Urban et al. (2018) suggest that the minimum dust emission (PM10) from the Mojave Desert currently ranges between 3–8 Tg/y. While *Atriplex polycarpa* and other plants associated with *Coccidioides* in the San Joaquin Valley and the Mojave Desert are not established on the Channel Islands (Junak et al. 2007), other *Atriplex* species do occur. Recent efforts, however, to detect *Coccidioides* in coastal soils north of Cambria (San Luis Obispo County near a Northern Elephant Seal rookery), near Ventura (Ventura County) and on Santa Catalina Island (several different inland and coastal ecosystems) have been unsuccessful (Antje Lauer, unpubl. data). Therefore, fugitive dust from mainland sources either in California or Baja California in Mexico (Laniado-Laborin 2007) appears to be a more probable exposure risk for marine mammals along the coast of California.

Although dust generated in the Mojave Desert and transported to coastal areas has not been investigated for the presence of *Coccidioides* arthroconidia, indirect evidence suggests that fugitive dust generated in endemic areas of

Table 4. *Coccidioides* prevalence in California Sea Lions (*Zalophus californianus*) that were part of this study across years (log-likelihood test; sex and age class pooled) 2013–2015, between sexes (year and age-class pooled, with Yates correction), and between age classes (years and sex pooled). Independent rather than joint comparisons were made due to sample size considerations; given that none of the comparisons were significant, no corrections for non-independence have been included. Statistical power was calculated post-hoc under assumptions of a moderate effect size of 0.3 and a small effect size of 0.1 to provide a reasonable range. The abbreviations n = sample size, df = degrees of freedom, G = G*Power, *P* = *P*-value.

Comparison	Categories	Percentage Positive	overall n	df	G	*P*	power (0.3)	power (0.1)
between years	2013	16.7	36	2	1.43	0.49	0.89	0.17
	2014	11.7	51					
	2015	8.2	49					
between sexes	female	10.3	87	1	0.007	0.94	0.94	0.21
	male	12.3	49					
between age groups	pup	18.2	22	3	10.02	0.02	0.85	0.14
	yearling	0	20					
	subadult/juvenile	15.9	44					
	adult	10.0	50					

the pathogen has caused disease elsewhere (Pappagianis and Einstein 1978; Flynn et al. 1979; Schneider et al. 1997; Fisher et al. 2012). Dust potentially carries *Coccidioides* arthroconidia to offshore islands and could therefore be a source of infection for pinnipeds and other marine mammals. Recently, Polymerase Chain Reaction (PCR) fragments of *Coccidioides* ribosomal DNA from dead California Sea Otters (*Enhydra lutris nereis*) found in coastal counties were shown to be 99% related to a 460 bp fragment obtained from soils west of Lancaster, California (98% query coverage, GenBank nucleotide database at the National Center for Biotechnology and Informatics [NCBI]: GenBank Accession # KY306699; Huckabone et al. 2015; Colson et al. 2017), which is consistent with the hypothesis that fugitive dust from the Antelope Valley of California is a source of exposure and possible infection with *Coccidioides* in traditionally non-endemic areas.

We detected no significant differences in exposure risk for California Sea Lions between 2013 and 2015. Because many adult females remain near the Channel Islands year-round, we anticipated an increased risk of pathogen exposure for these females relative to subadult and adult males, which roam to areas that are not as impacted by fugitive dust from the mainland (far out into the Pacific Ocean or further north), but our data show that both sexes had similar exposure risks (rates of seroconversion). When comparing age groups, however, we found that yearlings which made up about 16% of the sea lions did not show any evidence of seroconversion to the pathogen based on AGID assays. This led to the observed significant difference between age classes. A larger sample size may provide some insights as to whether older animals have higher risk of exposure to *Coccidioides* and if pups benefit from maternal antibodies for at least some time. We also expected to see a higher risk of exposure for animals stranding in counties closer to endemic areas of the pathogen, such as San Luis Obispo and Los Angeles counties, but instead we found no significant difference among locations. This could indicate similar risks of exposure across coastal counties or could instead reflect the migratory behavior of the animals meaning that stranding location may not be a useful proxy for location of exposure.

The dramatic increase in reported coccidioidomycosis incidence among humans residing in coastal California counties since 2016 is a concern (California Department of Public Health [CDPH] 2019. Infectious diseases by disease, county, year, and sex. CDPH. Available from https://data.chhs.ca.gov/dataset/infectious-disease. [Accessed 19 September 2019]) and should also alert animal conservationists to consider potential increases among wildlife populations, including pinnipeds (Appendix Figure 2). A comparison between locations in which sea lions presented with *Coccidioides* exposure and location in which humans presented with coccidioidomycosis could be attempted in the future with more expanded sampling and analysis of sea lion serum. A significant increase in *Coccidioides* titers in screened sea lions or other pinnipeds, such as Harbor Seals (*Phoca vitulina*) might serve as a warning for human exposure to the pathogen. Indeed, in 2017 and 2018, TMMC reported additional cases of coccidioidomycosis among California Sea Lions and MMC San Pedro identified several suspected cases that responded to antifungal treatment (pers. observ.). In addition, it is interesting to note that California Sea Lions are being used as sentinels for ocean and public health serving as indicators for domoic acid poisoning and other health concerns (Bossart 2011).

AGID is the most commonly employed serological test used to diagnose exposure to *Coccidioides* in humans and animals but has not been used on pinniped blood samples on a regular basis. The sensitivity of this test when performed on canine blood serum samples was determined to be 87% for immunoglobulin G and 46% for immunoglobulin M (Gunstra et al. 2019). The sensitivity and specificity of AGID assays are generally similar to antibody Enzyme Immuno Assays (EIAs) using infected canines (Holbrook et al. 2019). In another study investigating canines, AGID for IgG and IgM was

found to be 100% sensitive (100%) confirming results by histopathology (Johnson et al. 2003). Determining antibody titers can help distinguish acute from chronic disease; however, quantitative titration of antibodies does not always correlate with clinical disease in dogs or other animals (Greene and Troy 1995; Shubitz and Dial 2005; Shubitz 2007; Graupmann-Kuzma et al. 2008; Burgdorf-Moisuk et al. 2012). Therefore, AGID results should be evaluated with caution and results from ancillary diagnostic studies (e.g., from cytology, histopathology, culture, or PCR) should be included when available to confirm the diagnosis. Although this serology method has not yet been validated in sea lions or other marine mammals to correctly diagnose coccidioidomycosis, the test might serve as an indicator of exposure to the pathogen. According to Pappagianis and Zimmer (1990), the concentration of antibodies in sera from human patients generally decreases to undetectable levels for most patients with resolved infections. Therefore, patients with detectable anti-coccidioidal antibodies likely have recent exposure, illness or reactivation, or have chronic active disease.

Based from results from this and past studies, it is reasonable to assume that an AGID assay detects *Coccidioides* specific IgM in pinnipeds and indicates coccidioidomycosis in an early stage of the disease (acute infection). As disease progresses, IgM is replaced with IgG, which can indicate late or chronic disease process, or low levels may indicate acquired or waning immunity from previous exposure to the pathogen (Pappagianis and Zimmer, 1990). In our study, the titer of anti-*Coccidioides* antibodies in pinniped blood sera was not determined but would be necessary to distinguish actual disease (and the severity of it) from cell mediated immunity that indicates exposure to the pathogen (Pappagianis and Zimmer 1990). Paired serology studies would improve our ability to interpret titers (acute versus convalescent), but this method was beyond the budget of this study. Recently, Chow et al. (2017) developed an enzyme immunoassay (EIA) to detect *Coccidioides* specific antibodies in mammalian species in general by validating this method on dogs and mice. Neither method has been tried on blood sera from pinnipeds. A careful validation of the AGID and EIA as an indicator of both disease and exposure to *Coccidioides* would increase the confidence of using this method as a diagnostic tool for pinnipeds.

Because cell mediated immunity is the key mechanism of defense against coccidioidomycosis (Graupmann-Kuzma et al. 2008), early diagnosis of coccidioidomycosis, followed by treatment with an antifungal agent and supportive care that strengthens the immune system of an animal, should improve recovery from the disease. Delayed diagnosis of the disease poses the risk of dissemination, environmental contamination, unsuccessful rehabilitation, and potential death. For example, coccidioidomycosis was not considered as an underlying cause of stranding for three sea lions (one subadult female [case # 11977, stranded in Santa Cruz County] and two juvenile males (case #12549, stranded in San Mateo County, and case #11502, stranded in San Luis Obispo County) that were euthanized or died in treatment in the same year (2011 and 2012); all animals were IgM positive for *Coccidioides*. Approximately 1/3 of California Sea Lions with positive immunodiffusion assays (either IgM or IgG) in this study died during treatment or were euthanized.

Use of a quantitative titer in addition to AGID assays for IgM and IgG to distinguish acquired immunity to the pathogen from acute or chronic infection, particularly if the animal does not respond to treatment for more common causes of pneumonia (bacteria and parasites) and the exclusion of other causes of respiratory disease, may enhance detection of active infections with *Coccidioides*. This strategy will increase the chance for successful rehabilitation and avoid expensive and ineffective treatments with potentially hazardous side effects (Butler et al. 1964; Graupmann-Kuzma et al. 2008). The focus of this study was on blood serum from animals that presented with respiratory problems, but it is important to acknowledge that not all pinnipeds that present with respiratory issues are infected with *Coccidioides*. Future studies should continue to examine blood serum from all stranded animals and determine if respiratory problems are a good indicator to consider coccidioidomycosis as an underlying cause of stranding in pinnipeds as it is in human patients.

We plan to continue our work with pinniped blood sera to 2021 (under NOAA permit 19706), to relate seroprevalence or rate of detected cases of coccidioidomycosis to environmental changes, such as El Niño events (in 2015 and 2016), effects of future droughts, and increases in fugitive dust from the endemic mainland due to increases in soil disturbance and dust events. Analysis of this long-term dataset may provide correlations between human and pinniped exposure and possibly disease incidence, which could provide conservationists with an early warning of expected increases in strandings due to coccidioidomycosis. We are confident that these early efforts will help to establish a continuous monitoring project for disease incidence among stranded California Sea Lions, which are the most numerous rescued pinnipeds in the MMCs, as indicators for *Coccidioides* exposure risk for other marine mammals along the coast of California. Our data are an important baseline to help achieve this goal. Our work will contribute to improved outcomes in pinniped rehabilitation and conservation efforts by raising awareness of a disease that is often misdiagnosed and overlooked in regions that are not endemic for *Coccidioides* but may be impacted by fugitive dust emission from endemic areas of the pathogen. Furthermore, assessment of the health status of stranded marine mammals not only provides valuable information concerning care of these animals but also

provides information about existing and emerging health problems in wild populations and environmental health.

Acknowledgments.—We thank Terry Oubsuntia for performing all AGID assays. We also thank Dr. Claudia Jonah (Kern Medical Center, Public Health Laboratory) and Dr. Zed Mason (California State University, Long Beach) and his wife Helen Batchelor (MMCCLA San Pedro) for supporting our research. This work was supported financially by the California State University Council on Ocean Affairs, Science and Technology (COAST), NOAA Fisheries (permit #19706), and by the Louis Stokes Alliance for Minorities (LSAMP, National Science Foundation HRD-1302873).

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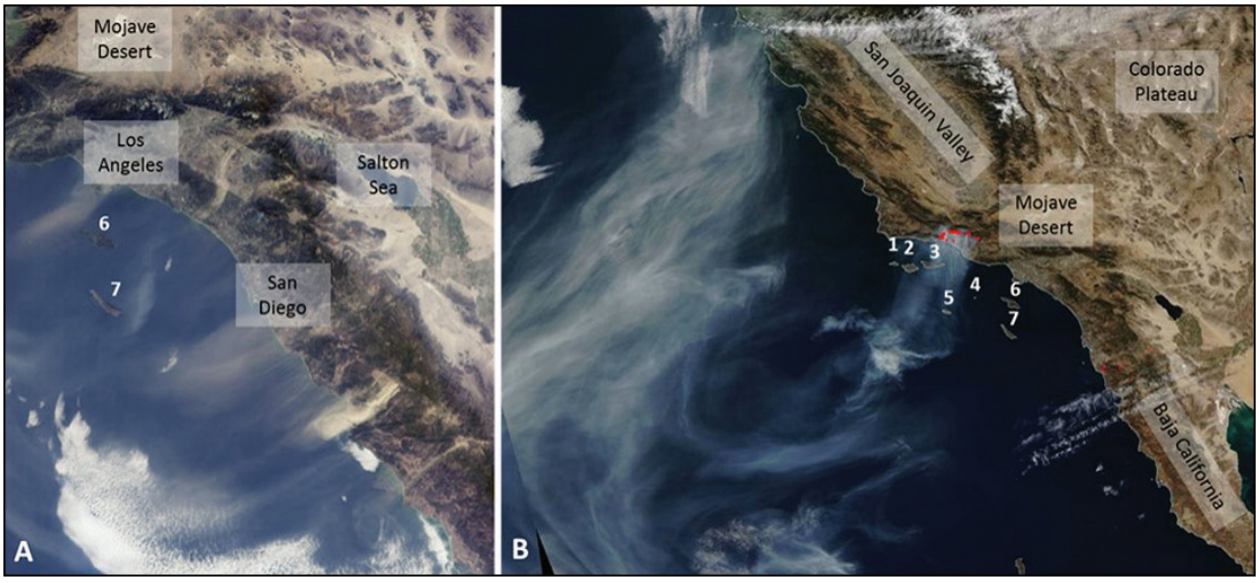
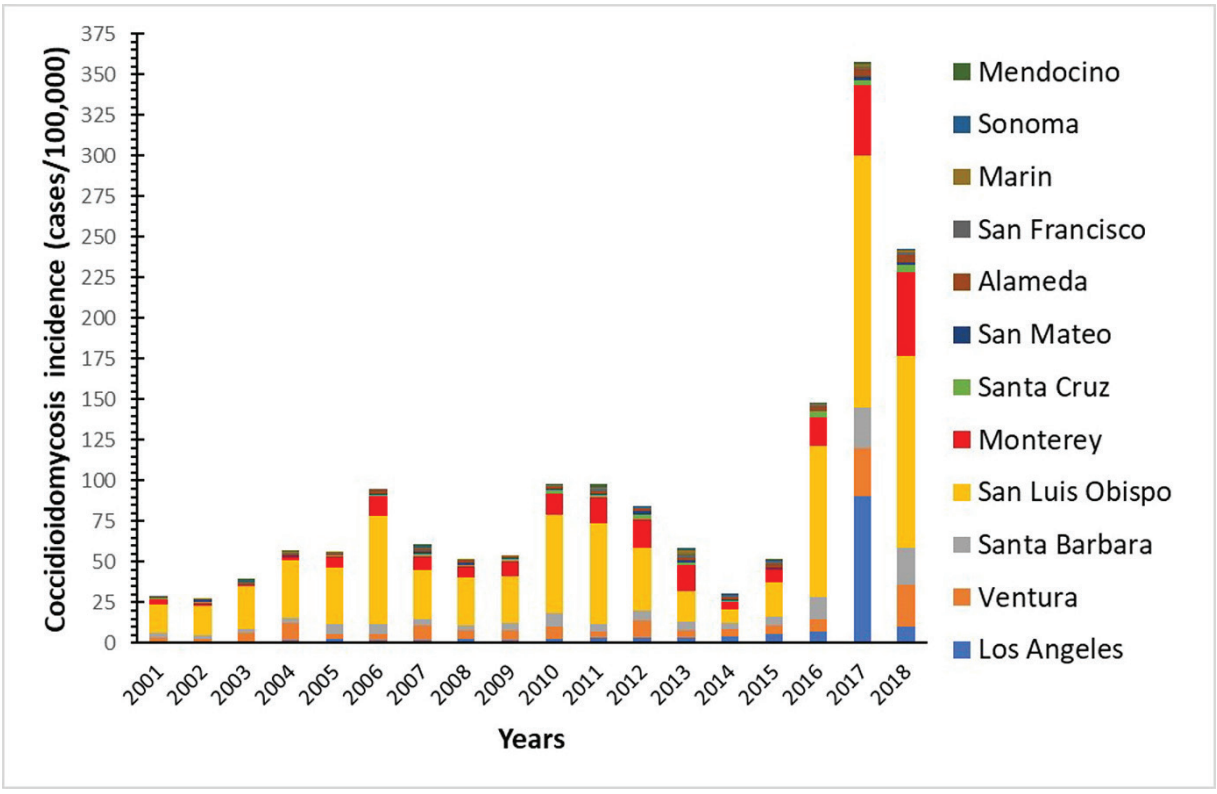
Appendix Table. Information about California Sea Lions (*Zalophus californianus*) diagnosed with *coccidioidomycosis* postmortem between 2005 and 2014 at The Marine Mammal Center (TMMC) in Sausalito, California. Age classes are based on straight length of body (Laake et al. 2016). Abbreviations for age classes are Y = yearling, J = juvenile, S = subadult, and A = adult, and for sexes are M = male and F = female.

Case #	Date	County	Age class	Sex	Result	Cause of death
6519	30 April 2005	San Luis Obispo	Y	M	died in treatment	disseminated coccidioidomycosis, malnutrition
6897	5 June 2006	Santa Barbara	J	M	died in treatment	disseminated coccidioidomycosis, malnutrition
7036	17 September 2006	San Luis Obispo	A	F	died in treatment	disseminated coccidioidomycosis, malnutrition
7830	13 August 2008	Santa Cruz	A	F	euthanized	severe coccidioidomycosis, domoic acid toxicity, malnutrition
8108	4 May 2009	Santa Cruz	A	F	died in treatment	disseminated coccidioidomycosis, domoic acid toxicity
9739	2 July 2010	San Luis Obispo	Y	F	died in treatment	peritonitis (unknown), malnutrition
9748	4 July 2010	Monterey	S	M	died in treatment	peritonitis (unknown)
9991	7 July 2011	Santa Cruz	S	F	died in treatment	disseminated coccidioidomycosis
10054	13 August 2011	San Luis Obispo	A	F	died in treatment	disseminated coccidioidomycosis, sepsis
10279	3 June 2012	Monterey	S	M	died in treatment	disseminated coccidioidomycosis, sepsis (perforation)
10630	21 April 2013	Monterey	J	M	euthanized	disseminated coccidioidomycosis, myocardinal infarction
10700	14 August 2013	Monterey	J	M	euthanized	disseminated coccidioidomycosis



Appendix Figure 1. (A) Example of dust transport from mainland California during Santa Ana conditions on 9 February 2002 (modified from NASA image, Multi-angle Imaging SpectroRadiometer [MISR] 2019). (B) Sparked by strong Santa Ana winds, inland dust and smoke from the Thomas Fire (13 December 2017) was carried several hundred miles into the Pacific Ocean (modified from NASA image, NASA Worldview Application operated by the NASA/Goddard Space Flight Center Earth Science Data and Information System [ESDIS] project). Actively burning areas were detected by Moderate Resolution Imaging Spectroradiometer [MODIS]; thermal bands are displayed in red. The numbers refer to Channel Islands of California. 1: San Miguel Island, which supports major California Sea Lion (*Zalophus californianus*) and Northern Fur Seal (*Callorhinus ursinus*) rookeries, 2: Santa Rosa Island, 3: Santa Cruz Island, 4: Santa Barbara Island, 5: San Nicolas Island (major California Sea Lion rookeries), 6: Santa Catalina Island, 7: San Clemente Island.



Appendix Figure 2. Reported incidence of coccidioidomycosis in humans residing in coastal California counties between 2001 and 2018. Counties are shown from south (bottom) to north (top; data obtained from: https://data.chhs.ca.gov/dataset/infectious-disease).