

Survey for Selected Pathogens and Evaluation of Disease Risk Factors for Endangered Hawaiian Monk Seals in the Main Hawaiian Islands

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Abstract: A recently reestablished and increasing population of Hawaiian monk seals in the main Hawaiian Islands (MHI) is encouraging for this endangered species. However, seals in the MHI may be exposed to a broad range of human, pet, livestock, and feral animal pathogens. Our objective was to determine the movement and foraging habitats of Hawaiian monk seals in the MHI relative to the potential exposure of seals to infectious diseases in near-shore marine habitats. We captured 18 monk seals in the MHI between January 27, 2004 and November 29, 2005, tested them for various infectious diseases, and then monitored the foraging movements of 11 of them using satellite-linked radio transmitters for the next 32–167 days. All seals tested negative for canine adenovirus, calicivirus, four morbilliviruses, phocine herpes virus, *Leptospira* sp., and feline and canine heartworm antigen/antibody. Six of the seals tested positive on complement fixation for *Chlamydomphila abortus* (formerly *Chlamydia psittaci*). Four seals demonstrated positive titers to *Sarcocystis neurona*, two to *Neospora caninum*, and two to *Toxoplasma gondii*. Fecal cultures showed approximately half ($n = 6$) positive for *E. coli* 0157, no *Salmonella* sp., and only one with *Campylobacter* sp. Satellite monitored seals spent considerable time foraging, traveling, and resting in neritic waters close to human population centers, agricultural activity, and livestock ranges, and sources of land-based water runoff and sewage dispersal. Consequently, Hawaiian monk seals in the MHI may be at risk of exposure to several infectious disease agents associated with terrestrial animals that can contaminate marine habitats from runoff along drainages and that are known to cause disease in marine mammals. Further, some seals overlapped substantially in their use of coastal habitats and several moved among islands while foraging and were seen on beaches near each other. This suggests that diseased seals could infect healthy conspecifics throughout the MHI.

Key words: Hawaiian monk seal, *Monachus schauinslandi*, Hawaiian Islands, leptospirosis, toxoplasmosis, pathogen pollution

INTRODUCTION AND PURPOSE

Infectious diseases can have substantial effects on marine mammal populations by directly causing mass mortality or morbidity, which increases susceptibility to predation or

Table 1. Tracking Periods for Hawaiian Monk Seals in the Main Hawaiian Islands in 2004

Seal ID	Deploy site	Deploy date	Age class	Sex	Track start	Track end	Days tracked
34150	Molokai	4/13/2004	Juvenile	M	13-Apr-04	27-Jul-04	105
34874	Kahoolawe	11/29/2005	Adult	M	29-Nov-05	16-Jan-06	54
34875	Molokai	6/9/2004	Weaned pup	F	9-Jun-04	22-Aug-04	74
34877	Molokai	4/12/2004	Adult	F	13-Apr-04	9-Jul-04	87
42675	Oahu	1/27/2004	Weaned pup	M	28-Jan-04	10-Jun-04	133
42677	Molokai	8/24/2004	Weaned pup	M	24-Aug-04	9-Dec-04	107
42678	Kauai	2/19/2004	Juvenile	M	20-Feb-04	19-Jul-04	149
42680	Kauai	2/14/2004	Adult	M	19-Feb-04	3-Jul-04	134
42681	Kauai	5/4/2004	Adult	M	5-May-04	17-Aug-04	104
42682	Kauai	7/5/2004	Adult	M	3-Jul-04	16-Dec-04	167
42683	Kauai	5/3/2004	Juvenile	M	4-May-04	11-Aug-04	99
42684	Oahu	2/23/2004	Adult	M	24-Feb-04	26-Apr-04	32

^aTotal tracking duration for Seal TT40 (PTT ID 42680 and PTT ID 42682) = 301 days.

other more debilitating diseases, and by inhibiting growth and development with corollary adverse affects on lifetime reproductive success (e.g., Conrad et al., 2005; Costas and Lopez-Rodas, 1998; Harwood and Hall, 1990; Honnold et al., 2005; Miller et al., 2002; Osterhaus et al., 1997, 1998; Raga et al., 1997; Stoddard et al., 2005; Thomas and Cole, 1996). Moreover, infectious diseases may have important influences on genetic structure and evolution of some species, particularly those with small populations (cf. Weber et al., 2000; Lehman et al., 2004).

In the Northwestern Hawaiian Islands (NWHI), the primary population of Hawaiian monk seals (*Monachus schauinslandi*) declined by 50% from the late 1950s through the mid-1970s. Consequently, the species was listed as *endangered* under the U.S. Endangered Species Act. The population decline has continued overall, though seal abundance appears to be increasing in the main Hawaiian Islands (MHI; Baker and Johanos, 2004). Seals in the MHI appear to be more robust (e.g., greater girth and length at weaning) than those in the NWHI, and the apparent increase in abundance in the MHI is encouraging for the species' overall status and vitality (Baker and Johanos, 2004).

Although the influence of disease on monk seal populations is not well understood, the Draft Recovery Plan for Hawaiian monk seals and the Contingency Plan for Hawaiian Monk Seal Unusual Mortality Events (Yochem et al., 2004) include infectious diseases among the threats to the persistence and vitality of the species. Of the 15 Marine Mammal Unusual Mortality Events (UMEs) reported for

other marine mammal species between 1992 and 2001 (Dierauf and Gulland, 2001: Table 1, pp 72–73), infectious diseases and biotoxins were the most common diagnoses. Baseline data on the prevalence of disease are needed to evaluate the potential population impacts of infectious agents (Thrusfield, 1995).

Trans-species contagion from domestic species or humans has been identified as one of three ways in which wildlife species may be exposed to emerging or resurging diseases (Miller et al., 2001). For example, pathogens traditionally associated with domesticated animals have been implicated in recent reports of transmissible diseases in marine mammals (e.g., *Brucella* sp., Ross et al., 1996; Jepson et al., 1997; *Mycobacterium bovis* and *M. tuberculosis*, Forshaw and Phelps, 1991; Cousins et al., 1993, 2003; Woods et al., 1995). Mainland freshwater runoff has been implicated as a risk factor for infection of threatened southern sea otters (*Enhydra lutris nereis*) with *Toxoplasma gondii* (Miller et al., 2002; Conrad et al., 2005), a protozoal parasite with felids as definitive hosts and a wide range of intermediate hosts (Hill et al., 2005). Seals that haul out and breed on coastal habitats and forage in nearshore marine habitats in the MHI may be exposed to a much larger suite of potentially debilitating diseases than seals in the geographically remote NWHI, owing to the ubiquitous presence of humans, pets, feral animals, livestock, and terrestrial wildlife in the MHI. Federal management of most of the monk seal habitats in the NWHI has also resulted in procedures to limit or prevent the introduction of exotic and invasive species. Several infectious disease agents

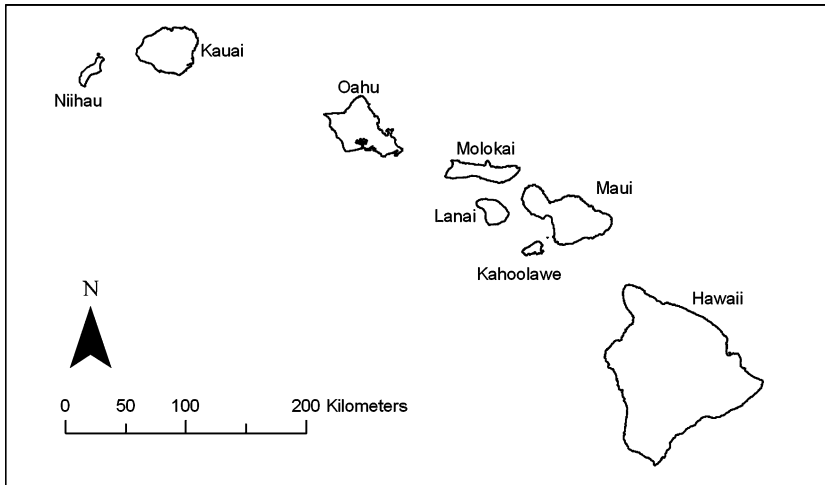


Figure 1. The main Hawaiian Islands (MHI).

that may affect monk seals (e.g., *Brucella*, *Leptospira*, *Toxoplasma*) are, however, ubiquitous in the MHI (Anderson and Minette, 1986). Recent modifications to the pet quarantine program eliminated the mandatory 120-day quarantine conditioned on satisfaction of several rabies vaccination requirements. Though the mandatory quarantine was primarily designed to prevent the introduction of rabies, it also was an important surveillance system for other serious infectious diseases. Its elimination may result in additional risks to monk seals.

Moreover, there is some risk of transfer of disease from seals in the MHI to those in the NWHI. The monitoring of seals born and tagged in the MHI and in the NWHI has demonstrated short- and long-term movements of seals among the island groups (Stewart and Yochem, 2004ab) and, on occasion, immigration to the MHI (Harting, 2002; Johanos and Baker, 2002).

Our goals in this study were to: (1) document the movements of monk seals in the MHI; (2) evaluate the risks of exposure of monk seals that use near-shore marine habitats to infectious disease that may be associated with human occupation of, and activities on, the islands; (3) consider the potential for conspecific spread of disease by movements of seals among the islands; and (4) consider options to mitigate the effects of existing and emergent diseases on this increasing important component of the monk seal population.

METHODS

Between January 27, 2004 and November 29, 2005, we captured 18 seals in the MHI (Fig. 1) with a hoop net and

then physically restrained and sedated each with an intravenous injection of diazepam (0.1–0.2 mg/kg; via the extradural vein which lies above the vertebral column). The handling and restraint techniques described here have been used with Hawaiian monk seals in the NWHI with no adverse effect (Baker and Johanos, 2002).

Within 2–10 minutes of sedation, blood, tissue (blubber and skin), fecal, and viral and bacterial swab samples (rectal, nasal, and genital) were collected using techniques previously described by Aguirre et al. (1999) and Aguirre (2000). Dorsal straight length (i.e., standard length) and axillary girth were measured to within 0.5 cm. Biomedical samples were processed within 2–5 hours of collection using protocols described by Aguirre et al. (1999), Aguirre (2000), and the 2000 Field Manual for Research on the Hawaiian Monk Seal [Anonymous, 2000, unpublished document, NOAA, SWFSC].

Analyses of hematology and serum and plasma biochemistry were done by IDEXX Laboratories (West Sacramento, CA). Bacteriological samples were refrigerated and shipped on blue ice (wrapped to prevent freezing) to the University of California Davis (Davis, CA) for evaluation. Fecal samples (fresh smears and fecal flotation) were examined for the presence of protozoal (e.g., *Giardia*) and helminth parasites by a veterinary technician at the Pacific Islands Fisheries Science Center. Serological tests for selected protozoa (*Sarcocystis neurona*, *Neospora caninum*, *Giardia lamblia* and *Toxoplasma gondii*; University of California Davis; Conrad et al., 2005) and bacteria (*Chlamydomphila*, *Leptospira* sp.; National Veterinary Services Laboratories [NVSL], Ames, IA) were performed. Testing of samples for *Leptospira* was performed using the microscopic agglutination test (MAT) for group antibodies

against *Leptospira pomona* antigen at a test dilution of 1:10 to determine if seals had been previously been exposed to serovars *pomona* (Pomona), *icterohaemorrhagica*, *grippotyphosa* (Moskva V), *autumnalis*, *ballum*, *canicola*, *grippe*, *hardjo*, *ictero*, *bataviae*, *pyrogenes*, *tarassovi*, *hebdomadis*, *sejroe*, and *szwajzak*. Threshold titers were considered positive at 1:100 for all serovars.

Specimens were tested for antibody presence of *Chlamydomydia abortus* (formerly *Chlamydia psittaci*) by micro and macro complement fixation (CF 1:10) at NVSL. Sera demonstrating a CF titer of IgG antibodies $\geq 1:20$ were considered evidence of prior natural exposure (OIE, 2000).

The Washington Animal Disease Diagnostic Laboratory (Pullman, WA) conducted serologic tests for canine adenovirus-1 (CAV-1) using virus neutralization test (VNT; threshold titers were considered positive at $>1:8$; Appel and Robson, 1973). The Laboratory for Calicivirus Studies (LCS, Corvallis, OR) used the VNT to test for evidence of group-specific antibody against San Miguel sea lion virus (SMSV) serotypes 1 to 17, walrus calicivirus 7420, feline calicivirus F-9, W-6 calicivirus, VESV A₄₈, primate calicivirus strain, mink calicivirus strain MV 20-3, cetacean calicivirus strain 041, bovine calicivirus BCV Bos-1, mystery pig disease calicivirus strain P42BN, canine calicivirus strain 731, *Oryctolagus* calicivirus, Hawaiian (temporary designation) calicivirus, McAll human calicivirus, cheetah calicivirus, fur seal herpes virus, and reptile calicivirus strain 002. The VNT was also used to test for antibodies to walrus adenovirus-1, human herpesvirus-2, walrus retrovirus, walrus enterovirus, and pinniped rotavirus. Threshold titers were considered positive at $\geq 1:8$ (Smith et al., 1977).

Serum was evaluated for antibodies to phocine herpesvirus 1 (PhHV-1), canine distemper virus (CDV), phocine distemper virus (PDV), dolphin morbillivirus (DMV), and porpoise morbillivirus (PMV) using microplate VNT (Saliki and Lehenbauer, 2001) by the Oklahoma Animal Disease Diagnostic Laboratory (Stillwater, OK). Threshold titers $\geq 1:8$ were considered positive. Tests were also done for feline heartworm antigen/antibody (IDEXX Laboratories, West Sacramento, CA) and heartworm microfilaria (*Dirofilaria immitis*; Difil-Test).

We attached a satellite-linked data recorder (SLDR; Wildlife Computers, Redmond, WA) to the dorsal pelage of each of 11 seals (5 adults [1 female, 4 males], 3 juvenile males, and 3 weaned pups [1 female, 2 male]) with quick-setting epoxy. We then monitored the geographic dispersion of the seals until the instruments failed, lost battery

power, fell off during the molt, or were removed. Geographic locations of the seals were determined several times each day by the Argos earth-orbiting satellite system and the Argos data collection location system (DCLS), described in detail elsewhere (e.g., Fancy et al., 1988; Stewart et al., 1989).

Geographic Information Systems (GIS) Mapping

To characterize areas of potential pathogen input (i.e., pathogen pollution; cf. Miller et al., 2002) from the terrestrial and freshwater to the nearshore marine environment, we collected spatial data sets detailing sewage spills, sewage outflows, agriculture land use, streams, and population centers. Those data and the seal movement data were imported into ArcGIS 8.3 (ESRI, Redland CA) and then geo-referenced and mapped with the Zone 4 (N) Universal Transverse Mercator (UTM) projection, using the WGS 1983 datum.

At-sea Movements and Habitat Use Estimates

The Argos DCLS uses several criteria to generate predictions about the accuracies of calculated locations and assigns an index of accuracy to each one. The best locations (LC = 1, 2, 3) are predicted to within a kilometer or less of the true transmitter location. Other locations are also made available to wildlife tracking community users (LC = 0, A, B, Z) though no predictions are made about their accuracies. Locations of LC = 0 may however be accurate to within several km or less. Consequently, we used only locations of LC ≥ 0 for describing the movements of monitored monk seals but we identified and eliminated some LC0 locations if rates of movements among locations exceeded those known to be reasonable for swimming seals.

To determine the spatial use of marine habitat by seals we imported the location data into a layer file and used the spatial analyst extension of ArcGIS to calculate kernel densities as an estimate of habitat use. Kernel density estimation is well suited for this type of analysis (Worton, 1987, 1989) as it is robust to small samples. For this study, we estimated habitat use by applying quartic approximation to a Gaussian kernel estimator. We calculated 95% and 50% probability distributions to represent the overall marine habitats used and the core habitats used (cf. Worton, 1989).

We supplemented the inter-island tracking data with opportunistic observations of the seals throughout the

MHI. We also examined an extensive database of observations of known seals (known minimum or exact age from tags or scars) in the MHI to assess movements of those seals among the MHI and between the MHI and the NWHI.

Pathogen Spatial Analysis

To characterize the potential seepage of hazardous farming chemicals (e.g., chlordane and dieldrin) and other pathogens relating to animal husbandry into freshwater streams and into coastal areas, we modeled the amount of overlap between agricultural sites (historical and current) and the stream systems of Hawaii. Agricultural land use and stream network maps were available for the islands of Kauai, Oahu, Maui, Molokai, Lanai, and Hawaii (State of Hawaii, Department of Business and Economic Development, Honolulu, HI). Agricultural land use maps represent areas that are used for animal husbandry (grazing, dairy, hog, and poultry), field crops (vegetables and melons, flowers, foliage, and grain), and orchards (banana, papaya, avocado, and others). We added an external buffer of 0.5 km to each agriculture polygon to account for runoff, and the line length of each stream within the agriculture polygon was summed to calculate the total overlap.

The discharge of raw or partially treated sewage into marine coastal waters enhances the risk of pathogen transmission to nearby fauna (e.g., Pearson and Rosenberg, 1978; Smith, 1996; Underwood and Chapman, 1996; Smith et al., 1999). Another source of contamination is non-point source pollution. Concentrations of *E. coli* and other fecal indicator bacteria in Hawaiian streams and storm drains routinely exceed Environmental Protection Agency (EPA) and World Health Organization (WHO) standards for recreational water quality and may be implicated in contamination of marine coastal waters (Fujioka, 2001). Various pathogens, including *Giardia* and *Cryptosporidium* (Johnson et al., 1995), have been detected at beaches or in near-shore waters just after sewage spills in the MHI. Incidences of raw and partially treated sewage spills in the MHI between January 2000 and December 2004 were provided by the Clean Water Branch of the Hawaii Department of Health. All spills ≥ 1800 L (500 U.S. gallons; $N = 689$) were imported into ArcGIS and plotted to determine spatial patterns in sewage spills.

We considered the limited spatial data available for *Leptospira* spp. occurrence and infections (a ubiquitous bacterial pathogen to humans and animals in freshwater streams and impoundments on all the MHI; Katz et al., 2002) in the

MHI, including surveys of animal vectors (i.e., brown rat *Rattus norvegicus*, house rat *Rattus rattus*, mongoose *Herpestes auro-punctatus*, and common house mouse *Mus domesticus*; State of Hawaii, Department of Health, Honolulu, HI). Those data focus primarily on residential areas on Oahu, making it difficult to extrapolate to the rest of the MHI.

RESULTS

We monitored the movements of 11 seals in the MHI for 32 to 167 days (Table 1). Most locations for all seals were in nearshore, neritic, marine habitats and within the 200-m depth contours surrounding the MHI or nearby banks (Fig. 2a–d). Several seals moved among the MHI. One juvenile male seal (42678) instrumented on Kauai traveled to the northwest and southwest coasts of Oahu (Fig. 2a,b). The adult males (42680/42682, 42681) that were tagged on the south coast of Kauai ranged extensively along the south and west coasts of Kauai and also traveled to Niihau. (Fig. 2a). After release, the adult male (34874) tagged at Kahoolawe swam overnight to Maui and then moved down the west coast of the island of Hawaii where it spent most of its time during the next 2 days. He then circumnavigated the island of Hawaii, returned to Kahoolawe, and then visited Lanai and Molokai before the tag failed. This adult male visited five of the MHI in less than 2 months.

Inter-island Movements

We summarized the inter-island movements of 73 seals that were either tagged as pups ($n = 23$), of minimum known age by tags and scars ($n = 29$), or were tagged as adult males being translocated from Laysan to various MHI in 1994 ($n = 21$). Of those, 26 (35.6%) animals were seen on islands other than where they were originally seen or to which they were translocated. Four of those were seals that we tracked in this study. Most seals that moved among islands were those that had been translocated to the MHI from the NWHI; 14 (67%) of 21 translocated males moved among sites.

Sewage Spills

From January 2000 through December 2004, 689 sewage spills were reported in the MHI; 630 occurred on Oahu, accounting for 105.6 million liters. The island of Hawaii had 23 spills totaling 6.7 million liters, Kauai had 13 spills totaling 1.6 million liters, and Maui had 22 spills totaling 1.1 million liters. No spills were reported for the other MHI.

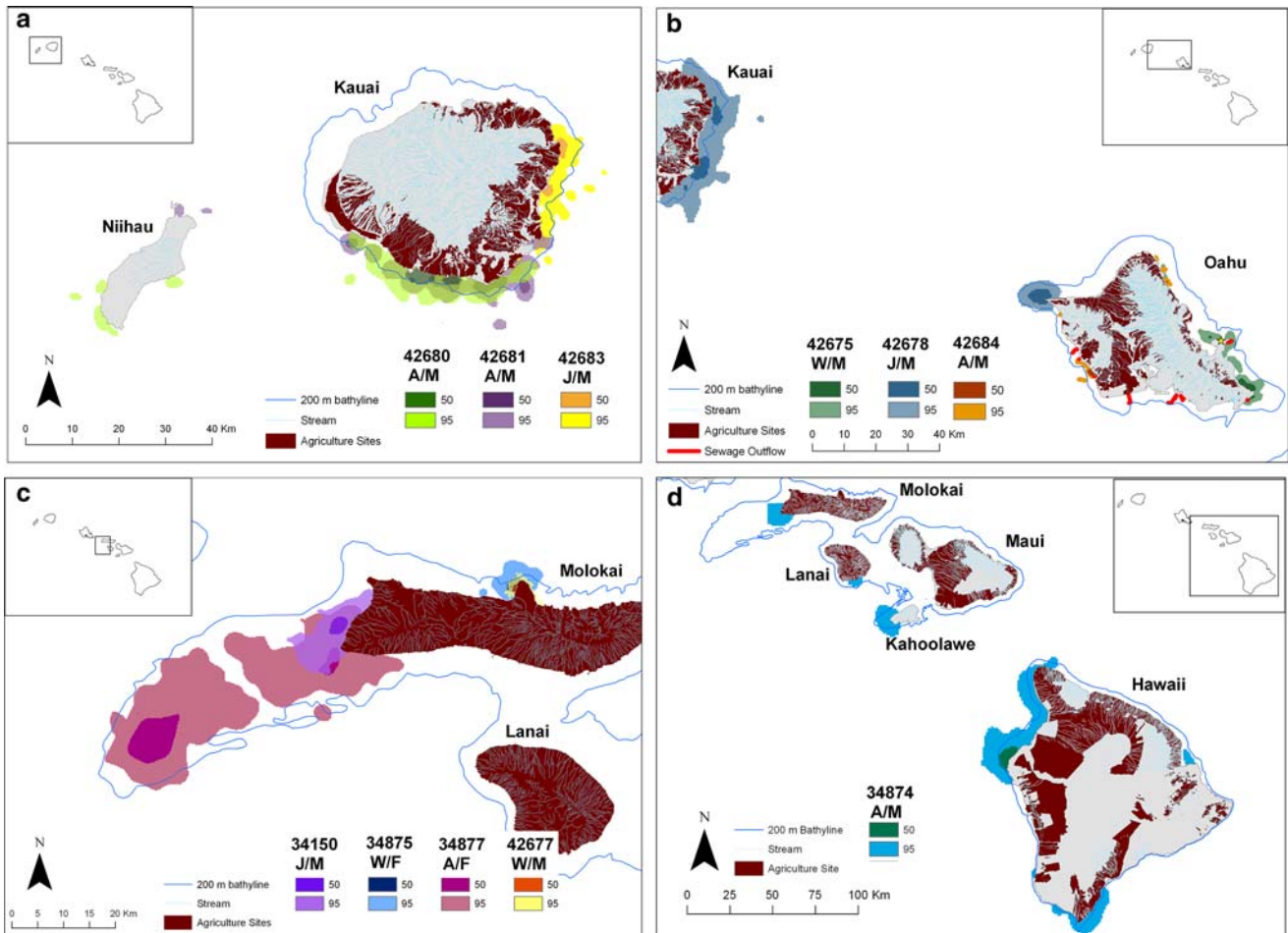


Figure 2. a–d: The calculated 95% and 50% probability distributions representing the overall habitats used by monk seals in the MHI. Location of streams and land used for agriculture are also indicated on the map. The age (A, adult; J, juvenile; W, weaner) and sex (M, male; F, female) classes of the seals are indicated.

The areas of most reported sewage spills were located near areas with large human populations (Fig. 3). On Kauai, most spill events occurred around Lihue and nearby Nawiliwili on the southeast coast of the island. Several areas with relatively greater occurrences of sewage spills were located on Oahu, including the area around Honolulu Harbor on the south-central coast, Kaneohe-Kailua on the eastern side, and along the northeast coast of the island. The coast around Lahaina was the only area of high sewage outflow detected on Maui. On Hawaii, most spills were located along the northern Kohala coast and near Hilo on the northeastern side.

Agriculture and Runoff

A total of 3953 km² of land were used for agricultural purposes in the MHI. Hawaii comprises the largest amount of agricultural lands (2331 km²) followed by Maui (622

km²), Kauai (383 km²), Oahu (364 km²), Molokai (164 km²), and Lanai (89 km²). Approximately 13,300 km of streams and rivers can be found in the main Hawaiian Islands with 7395 km (55%) passing within 0.5 km of an agricultural site. At least 50% (max 60%) of each island's total length of river passes through lands used for agricultural purposes. Nearly every freshwater outlet to the ocean in the MHI has at least one tributary that traverses an agricultural site (Fig. 2a–d).

Serological Tests and Cultures of Monk Seals for Pathogens

All seals tested negative for canine adenovirus, calicivirus, four morbilliviruses (canine distemper virus, phocine distemper virus, dolphin morbillivirus, porpoise morbillivirus), phocine herpes virus, *Leptospira* sp., and feline and canine heartworm antigen/antibody. Six seals tested posi-

tive on complement fixation for *Chlamydophila abortus* (formerly *Chlamydia psittaci*) (Table 2). Four seals demonstrated positive titers to *Sarcocystis neurona*, two to *Neospora caninum*, and two to *Toxoplasma gondii*. Although a presumptive diagnosis can be made based on rising antibody titers and clinical signs of disease, a single positive titer in an apparently healthy animal is not evidence of active infection but rather indicates previous exposure with or without clinical or subclinical disease. It may also be a false-positive resulting from cross-reactivity. Similarly, the presence of post-mortem lesions consistent with infection, not simply a positive titer indicating exposure, is necessary to implicate a pathogen as contributing factor in an animal's death. The fluorescent antibody assays used here for protozoa were developed and validated for sea otters (*Enhydra lutris*). Validation of these assays in Hawaiian monk seals is underway, including a comparison between Modified Agglutination Test and immunofluorescent assays of samples collected from a Hawaiian monk seal that died of disseminated toxoplasmosis (see below). *Sarcocystis neurona*, *Giardia lamblia*, and *Neospora caninum* organisms have yet to be identified in Hawaiian monk seals; tissues from a Hawaiian monk seal that died with high *Neospora caninum* titer (see below) are being evaluated for presence of protozoa to confirm serology. Fecal cultures from about half of the seals were positive for *E. coli* 0157. None tested positive for *Salmonella* sp. and only one tested positive for *Campylobacter jejuni* ss *jejuni*. The death of one monk seal in the MHI has been attributed to infection by *Toxoplasma gondii* (disseminated toxoplasmosis). Another dead seal had high titers to *Neospora caninum* (protozoal cysts in the lungs). Nonspeciatic *Leptospira* sp. was found in the kidney tissues of two other recently dead seals (Fig. 3) and may have caused their deaths (Honnold et al., 2005; [National Marine Fisheries Service (NMFS), unpublished data]).

DISCUSSION

Monk seals in the MHI forage, travel, and rest in near-shore waters close to human population centers (Fig. 3), agricultural activity and livestock ranges (Fig. 2a–d), and sources of land-based water runoff (Fig. 2a–d) and sewage dispersal (Fig. 3). Monk seals have also been reported to enter marinas, streams, and coastal lagoons and estuaries [NMFS, unpublished data]. Consequently, seals in the MHI are at risk from exposure to several key infectious diseases

associated with terrestrial animals that contaminate marine habitats from runoff along natural drainages or through sewage pipes (i.e., *Toxoplasma gondii*, *Sarcocystis canis*, *Leptospira* spp., *Brucella*, *Salmonella*). Survival of potentially pathogenic viruses and bacteria in seawater varies among organisms and with water temperature, up to 10 days or more in laboratory settings (Wait and Sobsey, 2001).

Direct Risk of Pathogen Exposure

Cats are ubiquitous definitive hosts of *Toxoplasma gondii*. The oocytes of the parasite that cats shed in feces are robust and may survive for long periods in terrestrial and aquatic environments (e.g., Conrad et al., 2005; Dubey et al., 2003; Wallace, 1973). In the MHI, this protozoan is prevalent in feral cat populations on Oahu and Hawaii, at least, and has been reported as an important pathogen affecting several terrestrial birds (Wallace, 1973; Work et al., 2000, 2002). Moreover, one adult male seal died of disseminated toxoplasmosis at Kauai in 2004 (Honnold et al., 2005; [NMFS, unpublished data]). Another seal died of myocarditis with protozoal cysts in the lungs on the north shore of Oahu in 2005. The seal at Kauai was apparently resident there and was known to often visit harbors and estuaries. It was apparently infected by ingesting free-floating oocytes directly or secondarily through consumption of oocyte-contaminated prey. The most likely source of the infective states is from cat feces (domestic or feral) at beaches or oocyte-contaminated soil being washed into the ocean along natural watershed drainages or sewage pipes (cf. Conrad et al., 2005; Miller et al., 2002).

Leptospirosis is among the most widespread zoonoses on Earth (WHO, 1999). The source of infection in humans and most species is direct or indirect contact with the urine of infected animals. Its prevalence is substantially greater in warmer climates (Everard and Everard, 1993). In the United States, the prevalence of these bacteria is highest in the MHI (Katz et al., 2002). Surveys of the small mammal populations indicate that rodents and mongoose are its principal vectors in Hawaii. Outbreaks of leptospirosis have generally occurred just after periods of hot, dry weather, when leptospire multiply in freshwater ponds or rivers. Some outbreaks have also occurred just after flooding from heavy rains. This is a cycle in Hawaii that might cause increased susceptibility of monk seals to infection with leptospirosis, highlighting the importance of this pathogen as a reemergent disease. Evidence implicating *Leptospira* sp. in the death of monk seals on the MHI is contradictory.

Table 2. Results of the Pathogen Screening Conducted on Hawaiian Monk Seals in the Main Hawaiian Islands

Seal ID	Island	Sex	Size	SN	Phocine Herpesvirus		<i>Chlamydia psittaci</i>	Titer	<i>Sarcocystis neurona</i>	Titer	<i>Toxoplasma gondii</i>	Heartworm Difil test	<i>E. coli</i> 0157 spp.	<i>Salmonella jejuni</i> ss <i>jejuni</i>	<i>Campylobacter jejuni</i> ss <i>jejuni</i>	Fecal cultures
					1	2										
42682	Captive	M	A	<4				40	40	40	n/a	n/a	+			<i>Vibrio cholerae</i> ative, <i>Edwardsiella tarda</i>
42680	Kauai	M	A	<4				<40	40	40			+			
R5AY	Kauai	F	A	<4	+			<40	<40	<40	n/a	n/a	n/a	n/a	n/a	
42681	Kauai	M	A	<4	+			<40	<40	<40			+			
34877	Molokai	F	A	<4				1:40	1:40	<1:40	<1:40		n/a	n/a	n/a	
34877	Molokai	F	A	<4				1:40	1:40	<1:40	<1:40		+			Hemolytic <i>E. coli</i> , <i>Vibrio cholerae</i> ative, <i>Vibrio alginolyticus</i>
RY30	Molokai	F	A	<8				<1:40	<1:40	<1:40	n/a		+			
TF20	Molokai	M	A	<4				1:160	<1:40	<1:40	n/a		n/a	n/a	n/a	
TF20	Molokai	M	A	<4				<40	<40	40	n/a		n/a	n/a	n/a	
T34M	Oahu	M	A	<4	+			<40	<40	<40	<40		+			<i>Vibrio cholerae</i> ative, <i>Edwardsiella tarda</i>
RIAQ	Kauai	F	S	<4	+			<40	<40	<40	n/a		n/a	n/a	n/a	
R2AU	Kauai	M	S	<4	+			<40	<40	<40	n/a		n/a	n/a	n/a	
42678	Kauai	M	S	<4				<1:40	<1:40	<1:40	<1:40		+			<i>Vibrio cholerae</i> ative, <i>Pleisomonas shigelloides</i> ative
42683	Kauai	M	S	<4	+			<40	<40	<40	<40		+			
RM34	Hawaii	M	J	<4				<40	<40	<40	n/a		n/a	n/a	n/a	
RM34	Hawaii	M	J	<4				<1:40	<1:40	<1:40	n/a		n/a	n/a	n/a	
RH40	Kauai	M	J	<4				1:160	<1:40	<1:40	n/a		n/a	n/a	n/a	
34150	Molokai	M	J	<4				<40	<40	<40	<40		+			<i>Edwardsiella tarda</i> , <i>Vibrio cholerae</i> ative, <i>Vibrio parahemolyticus</i>
42675	Oahu	M	J	<4				<40	<40	<40	<40		n/a	n/a	n/a	
34875 ^c	Molokai	F	W	6.0				n/a	n/a	n/a	n/a		+		+	<i>Campylobacter jejuni</i> ss <i>jejuni</i>
42677	Molokai	M	W	<4				<1:40	<1:40	<1:40	n/a		+			

^aFour seals were sampled twice. All seals tested negative for canine adenovirus, calicivirus, morbillivirus (canine distemper virus, dolphin morbillivirus, porpoise morbillivirus), *Leptospira* (ative at 1:200) and feline heartworm antigen/antibody. Repetition of seals indicates multiple capture and sampling events.

^bSeals instrumented with SDRs in this study.

^cA complete serosurvey was not possible for seal R115.

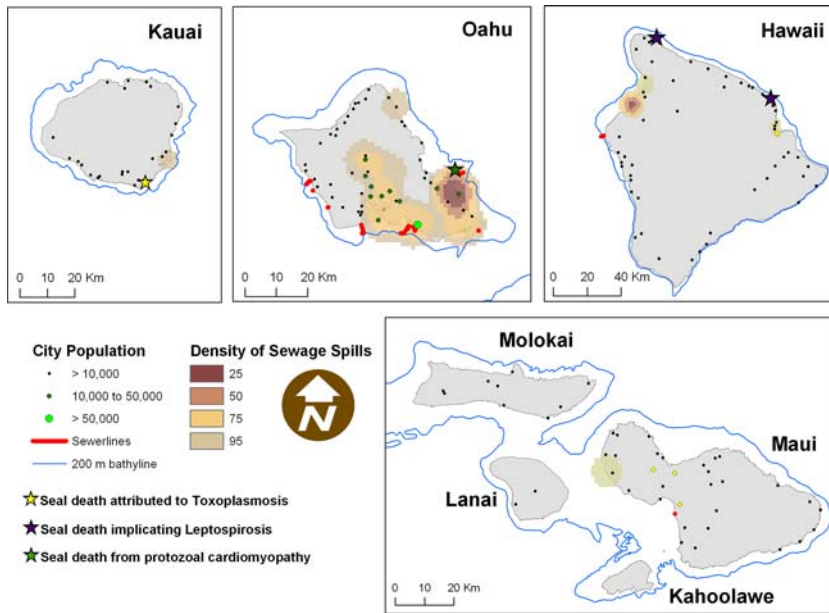


Figure 3. The distribution and population of major cities, sewage outflows, and sewage spills in Hawaii. The density of sewage spills is a probability distribution of where spills occur. The stars represent locations of seals whose deaths were due to toxoplasmosis (yellow), protozoal cardiomyopathy (green), or suspected of being linked to leptospirosis (purple).

Two recent deaths of monk seal pups are known cases of *Leptospira* infections as detected by real-time PCR, dark-field microscopy, and fluorescent antibody assays [NMFS, unpublished data]. Both of those pups were born and nursed near a stream outflow. Neither was seropositive for known pathogenic serovars. One had significant myocarditis of unknown origin. Kidney tissue positive to *Leptospira* (fluorescent antibody test) was recovered from another weaned male pup that died in Kapania, HI though those serum tests to antibodies of all *Leptospira* serovars at the California Animal Health and Food Safety Laboratory (University of California Davis) were all negative [NMFS, unpublished data, 2004].

Stoddard et al. (2005) documented susceptibility of phocid pinnipeds to the bacteria *Salmonella* and *Campylobacter* in contaminated coastal waters. Fenwick et al. (2004) suggested that *Salmonella* spp. may be cycling between New Zealand sea lions and feral pigs in the Auckland Islands, perhaps as a result of human waste contamination of the marine environment. *Salmonella* spp. and *Klebsiella pneumoniae* bacteria were isolated from New Zealand sea lion pups afflicted with systemic bacterial infections that were the primary or contributing cause of death in the pups (Duignan et al., 2003). Though no monk seals have yet been unequivocally diagnosed with disease caused by these bacteria in the MHI, there are sporadic reports of positive fecal cultures for *Salmonella* in about a third of the weaned pups sampled at the NWHI (adult prevalence closer to 1%) and a similar proportion of weaned pups brought to oceanariums for care

from 1985–1994 [NMFS, unpublished data]. Continued monitoring of the monk seals for these bacteria is essential because of the frequent expulsion of untreated waste water into the nearshore waters of the MHI and the documented impacts of these bacteria on marine mammals generally.

West Nile virus (WNV) is an emerging threat to wildlife everywhere (Campbell et al., 2002), including phocid pinnipeds. Hawaiian monk seals and other phocids are susceptible to infection and mortality, as demonstrated by the death of one monk seal at SeaWorld San Antonio, by antibodies to the virus detected in other seals there (Dalton et al., 2004), and by reports of fatal WNV neurologic disease in a captive harbor seal (del Piero et al., 2006). Post-mortem findings for the monk seal included non-suppurative encephalitis and myocarditis suggestive of viral etiology. Serologic testing for WNV in this seal was performed at the Texas Veterinary Medical Diagnostic Laboratory and the New York State Veterinary Medical Diagnostic Laboratory, and both institutions reported positive titers at 1:40 for WNV; serologic tests for canine and phocine distemper were negative. Kilpatrick et al. (2004) quantitatively modeled the risk of WNV being introduced to the Hawaiian Islands through various pathways (e.g., migratory birds, mosquitoes on airplanes) and demonstrated a substantial risk through human-transported mosquitoes and birds. Considering the likely eventual introduction of WNV and the occurrence of monk seal haul-outs near potential breeding habitats of virus-bearing mosquitoes, this disease could substantially affect the MHI population of seals.

Risks of Disease Transmission among Seals

Contact rates are critical when looking at the possibility of disease transmission and, though monk seals often appear solitary when ashore on the MHI, they do interact with each other when at sea and they also occur together ashore at some sites in the MHI. This suggests the potential for diseased seals to infect other seals in the MHI. Indeed, four of the seals that ranged and foraged along the coast of Kauai had substantial overlaps in habitat use of coastal marine habitats and at times were observed on beaches together. Satellite tracking and population monitoring studies have demonstrated that seals commonly move among the MHI, sometimes within a few hours, which increases the opportunity to spread disease.

Resights of flipper-tagged or otherwise identifiable (e.g., scar patterns) seals also indicate that Hawaiian monk seals move among the NWHI, among the MHI, and, rarely, from the NWHI to the MHI. Only one seal has been observed to move from the MHI to the NWHI (i.e., to Nihoa). That seal was initially translocated to the MHI from the NWHI. Overlap and interaction between seals from the NWHI and the MHI may be more common than data indicate, particularly at the intermediate islands of Necker and Nihoa which have not been well studied. We think that future studies should determine how those intermediate areas might bridge the colonies at the NWHI and the MHI, and perhaps affect transmission of diseases between the two island groups.

Pesticide Runoff

Concentrations of certain organic pesticides (e.g., chlordane and dieldrin) in Hawaii are among the highest in the United States (Bevenue et al., 1972; Tanita et al., 1976; Schmitt et al., 1990; Hunter et al., 1995; Brasher and Anthony, 2000). Organochlorine pesticides are heavily and widely used in Hawaii's urban areas to control termites and mosquitoes (Yates and Tamashiro, 1990). Those compounds can enter the aquatic environment from a variety of sources, including industrial and municipal effluents and agricultural and urban non-point source runoff (Brasher and Wolf, 2004). The extensive and complex watershed system in Hawaii transits virtually all agricultural areas and generally flow less than 16 km before reaching the coast potentially providing a relatively short and direct route for flushing agricultural chemical runoff into near-shore waters (Oki and Brasher, 2003). Both intermittent and perennial

streams may acquire water along some reaches and lose water along other reaches depending on local geohydrologic conditions. Consequently, both ground water and surface water runoff can contribute contaminants to stream systems and eventually near-shore waters in the MHI (Lopes and Furlong, 2001).

The main concern about exposure of monk seals and other wildlife to these chemicals is not direct toxicity but that they may cause sublethal effects by immunosuppression, consequently increasing morbidity and mortality risks when exposed to infectious viruses and bacteria (e.g., Ross et al., 1996; van Loveren et al., 2000). These organochlorines have been linked to a range of other effects, including biochemical and physiological changes and behavioral changes, reduced fecundity, morphological deformations, and endocrine disruption (Murty, 1986; Madhun and Freed, 1990; Kavlock et al., 1996; Colborn and Thayer, 2000). Whereas the presence of these chemicals in the nearshore environment has been documented, their tissue levels and potential impacts on seals in the MHI are not known. This is a critical information gap for the conservation of the species.

CONCLUSIONS

Hawaiian monk seals in the MHI are at risk of exposure to several debilitating diseases related to human activities. The seals use near-shore marine habitats when foraging and they haul out on beaches near sources of pathogen pollution associated with human population centers, sewage spills, and terminal watershed outfall. Of 12 dead monk seals that have been thoroughly necropsied in the MHI since 1996, the deaths of four are known to have been related to infectious disease. One was attributed to an infection of *Toxoplasma gondii*. Another seal died of a protozoal cardiomyopathy, and *Leptospira* sp. has been implicated in the deaths of the other two. Those pathogens are associated with terrestrial animals or with pathogen pollution from terrestrial runoff into near-shore marine environments. The intensity and virtually certain persistence of most of these human activities and severely limited options for eradicating correlative diseases and vectors (e.g., domestic and feral cats, livestock, wild pigs), constrain options for managing disease in the increasing monk seal population in the MHI. We think that proactive measures should focus on: (1) continued and improved infectious disease surveillance; (2) determining the levels of

organochlorines and other anthropogenic compounds in, and their potential impact on, monk seals; (3) rapid response and treatment of infected seals; and, perhaps (4) selective vaccination of free-ranging seals to selected pathogens. Management of existing and emerging diseases in monk seals could be complicated by difficulties in detecting the occurrence of the disease in wild seals and implementing standard disease control measures in free-ranging animals. Further complications could stem from overlapping jurisdictions and diverse mandates of state and federal agencies, as well as public resistance to disease prevention or response measures that are perceived to be harmful to a publicly owned and highly treasured resource. We recommend that a standardized, but vigilantly adaptive, long-term monitoring program of health and disease of Hawaiian monk seals be further developed and implemented in the MHI as an important element in the conservation and management of this greatly endangered species.

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