

Hawaiian Monk Seal: Diseases and Vaccination

by

Andrea Chernov

MARS 6920-1

April 28, 2008

Justification

Diseases have severely impacted on several marine mammal populations. Most notable is the morbillivirus epizootic of the Mediterranean striped dolphin between 1990 and 1991, that killed at least 1,000 animals (Aguilar and Raga 1993). Mediterranean pinnipeds, particularly monk seals and harbor seals, were found to be susceptible to morbilliviruses, though no such outbreak had been seen (Osterhaus et al. 1992). In 1997, an African population of the endangered Mediterranean monk seals were devastated by morbillivirus- of the 300 monk seals, at least half died of a confirmed morbillivirus outbreak (Osterhaus et al. 1997, van de Bildt et al. 1999); another monk seal was found dead of morbillivirus infection a year later on the West coast of Africa (van de Bildt et al. 2001).

Hawaiian monk seals are a highly endangered pinniped found in the Hawaiian Island chain (Aguirre et al. 2007, Nielsen et al. 2005) whose numbers have been declining steadily over the years (Nielsen et al. 2005). So far, Hawaiian monk seals have not been affected by morbillivirus (Aguirre et al. 2007). However, Hawaiian monk seal cell lines were found to be highly susceptible to morbillivirus (Lu et al. 2003) and the disease could thus wreak havoc on the populations. Diseases found in the Hawaiian monk seal population include leptospirosis, *Brucella*, *Chlamydomphila abortus* (Aguirre et al. 2007), hepatitis (Yantis et al. 2003) and *Toxoplasma gondii* (Aguirre et al. 2007, Honnold et al. 2005), the last of which has been confirmed fatal (Honnold et al. 2005). The overall effects of these diseases on the population have not been expounded upon in the literature, though all have potentially harmful effects.

To support Hawaiian monk seal conservation efforts, a reduction of potentially harmful diseases must be carried out. Disease monitoring must be conducted so as to assess the overall population health and to see if and when various diseases, especially morbillivirus, enters the

population. This health assessment will allow researchers to monitor the effects of other potentially harmful diseases already present within the population. Development of vaccines must also be conducted in order to vaccinate individuals against all potential diseases that individuals could contract. Vaccines should be administered to individuals in a captive maintenance program, where post-weaned pups are maintained for a period of time in an enclosure (Gilmartin et al. 1986).

Several alternative and null hypotheses exist for this research. Alternative hypotheses include 1) diseases will have major effects on the Hawaiian monk seal population, 2) diseases will have variable impacts on different age classes, between the sexes, and on different colonies, 3) these diseases can be routinely monitored via standard serological and virology tests for marine mammals, 4) vaccines can be developed for the population for the appropriate diseases, and 5) the vaccines will be effective against the intended disease. Null hypotheses include 1) various diseases will have little to no effect on the monk seal population, 2) death due to disease will not affect age classes, either sex, or different colonies, 3) diseases cannot be routinely monitored with any sort of efficiency, 4) vaccine development will take decades, if it happens at all, and 5) vaccines will be ineffective against diseases of Hawaiian monk seals.

Objectives

- Monitor the overall health of the Hawaiian monk seal population, creating two catalogues of all diseases that have both affected and not affected the population, allowing for the proper vaccination development and administration to the population.
- Monitor death rates attributable to disease, creating a catalogue of those diseases affecting population numbers, providing crucial information on which diseases need priority in the vaccination schedule to decrease the current death rate.

- Determine the effects of disease on age class structure, sex dynamics, and colony structure to focus vaccination on these sections of the general population
- Develop vaccines for those diseases currently affecting monk seals so as to control the impact on the population as well as decreasing the number of infections and possibly the number of deaths due to the pathogen.
- Develop vaccines for those diseases with the potential to wreak havoc on the population but have not infected individuals to prevent an outbreak. This would help prevent mass strandings of animals from the introduction of previously unknown threats.
- Population vaccination with continued disease monitoring to determine vaccine effectiveness, providing a vaccination success rate as well as data on any emergent diseases not considered previously.

Methods

Potential Hawaiian monk seal diseases will be identified through a literature review of diseases affecting pinnipeds worldwide. Population health monitoring and the creation of two disease catalogues will be done through necropsy in conjunction with a captive maintenance program conducted by NOAA for a period of two years. Effects of disease on the population structure will be monitored through age and sex determination dead seals. Effects of disease on different colonies will also be established by determining the death rate due to disease.

Necropsy will be performed on all dead-stranded animals. Blood and organ tissue will be submitted to appropriate labs on the mainland United States for analysis. Formalin-fixed tissues will be submitted for immunohistochemical staining. Body condition, including blubber thickness and injuries, and lesions of internal organs will be noted. Sex and colony affiliation

will be noted to assist in establishing disease effects on these parameters. Age will be estimated using average body measurements.

All animals in the captive maintenance program will be serologically tested for various diseases when first brought into the program. Estimated age, sex and colony affiliation will be noted to assist in determining the effects of diseases in these three categories. Animals will be visually monitored for signs of illness, at which time blood will be taken and analyzed through laboratory analysis. All animals found positive will be isolated then treated. If the animal recovers, it will be vaccinated, tagged, and released after a set amount of time. If an animal dies, a necropsy will be performed using the same protocol for dead-stranded animals.

At the end of the two years, two catalogues will be created- one of pathogens and antibody titers found and one of those pathogens and antibody titers not found. Diseases affecting monk seals will be identified via laboratory testing in mainland labs, including serology tests and immunohistochemical staining. Serological testing will produce antibody titers, which indicate previous exposure to the pathogen. Positives on any or all of these tests means the disease will be placed in the first catalogue. If all tests performed for a disease come back negative, that disease will be placed in the second catalogue.

A student t-test will be administered to determine if there is a significant difference at the $P > 0.05$ level between diseases of monk seals. If there is a significant difference, vaccine development for those diseases will take priority and will be given first in the vaccination schedule. All other vaccines will be given according to significance of the t-test results. The second catalogue and a comprehensive literature review will determine which non-present diseases will have vaccines. Those diseases which the literature has deemed highly damaging to

natural populations will have vaccines. These vaccines will be given first in the vaccine schedule. Effectiveness will be determined through continued disease surveillance of the population, which will reveal if members of the population have been introduced to a particular disease not found in the natural population previously. Disease prevalence between non-vaccinated animals and vaccinated animals will be compared using a one-tailed t-test, with significant difference at the $P < 0.05$ level determining effectiveness.

Antibodies from positive blood serum will be used to create vaccines for diseases already in the population. Vaccines for non-present diseases will be created through antibodies found in the blood serum of other pinniped species, primarily the Mediterranean monk seals; those diseases which the literature deems highly catastrophic for natural populations will be focused on first. Vaccines will be administered during the captive maintenance program and animals will be monitored for the development of any diseases. All animals will be tagged prior to release to monitor post-release survival and vaccine effectiveness. Captive management records will indicate tag number, vaccination record, release date, and other health factors for each animal.

Vaccinations and tags will also be given to animals found hauled out on beaches. Females with pups will be avoided to minimize pup abandonment. Morphometrics, sex, and blood samples will be taken at the same time. Blood samples will be tested for various diseases as part of disease monitoring of the natural population. The vaccination schedule will focus on those age categories and colonies that appear to be most affected by disease. Vaccination schedules will also focus on the sex that is seen to be most affected by disease.

On-going disease monitoring of natural populations will determine effectiveness of vaccines and if new vaccines need to be developed. Disease monitoring will be performed via

animals released from the captive maintenance program, hauled out animals that were vaccinated and animal necropsy. Tagged animals seen hauled out will be monitored serologically for any diseases. Necropsy will be done using the protocols developed during the catalogue formation stage. If the animal was tagged, then the tag number will be compared to vaccination records to establish vaccination history. A t-test will also be administered to test significance between vaccinated and non-vaccinated monk seals at the $P < 0.05$ level.

Laboratory results from vaccinated and non-vaccinated individuals will be compared to determine effectiveness of vaccines and the need for the development of new vaccines. Diseases found to be newly affecting the population will be added to the catalogue. Ineffective vaccines will be noted and new vaccines will be formulated.

Results

At the end of the first two years of this study, two separate catalogues will have been created. The first catalogue will contain a list of those diseases affecting monk seals; the second will be of those diseases not currently found in the monk seal population. It is expected that certain diseases will be more prevalent in the population than other diseases. Hawaiian monk seals are expected to test negative for morbilliviruses and to do so post-vaccination. However, this study could be the first to report consistent positives of diseases, like morbilliviruses, that have previously been unknown to the population.

Vaccine administration will most likely be determined by the age class and/or sex, as disease differences between colonies are expected to be inconclusive at the end of this study. Colony differences are expected to be inconclusive as translocation is used in monk seal management, as discussed by Littnan et al. (2007). Any translocation that occurs could possibly

transmit diseases between colonies, especially if the animal was a disease carrier without researchers knowing it. Sex may or may not be significant. If there is a significant pattern (e.g. seeing more males die from disease than females), then vaccine administration will also go by sex. More than likely, any patterns in sex difference will be inconclusive due to small sample size and this factor will have to be studied more carefully in the future.

The vaccinations developed during this research are expected to be both effective and ineffective at disease prevention. Literature about the development of vaccines for marine mammals has not been found, so this would be the first known attempt at developing vaccines for diseases specifically affecting marine mammals both here in Hawaii and worldwide. More than likely, many of the vaccines for diseases present in the population will be ineffective. Any of the vaccines that are found to be non-effective will cease to be used; new vaccines will be created and evaluated for effectiveness. Those vaccines determined to be effective will continue to be administered to hauled-out animals and pups in the captive maintenance program. The effectiveness of some of these vaccines may be inconclusive. This may be because the animals with a particular vaccine died of a different disease or animals with that vaccine were not seen again. If that animal is not seen again, then researchers would not know if that animal survived or died; if it died, we would not know what it died of.

The effect of vaccines for non-present diseases may be inconclusive at the end of this study as these diseases will hopefully continue to be absent from the population, although at least one new disease is expected to be found during the post-vaccination period. Currently, no literature on the transmission of diseases from migrating species (e.g. the Humpback Whale) to local species has been found. Therefore, it is possible for animals like the Humpback Whale to bring in new diseases to the population, which would test a certain number of the vaccinations

created. This would also provide results for post-vaccination population monitoring, as any new diseases will be picked up through various testing methods.

Contributions

Recommendations for future research and conservation directions will be made based on all results. If the vaccines prove ineffective at disease prevention, even after several redevelopments, other routes must be considered to stop the spread of disease. Any vaccines proven effective should be used as a baseline for the creation of vaccines for other marine mammal species in other parts of the world.

The creation of a disease catalogue allows researchers and conservationists to determine the impact of certain diseases on the Hawaiian monk seal population. It can also guide all parties involved to appropriate conservation tactics that could help in preventing diseases that Hawaiian monk seals have not been exposed to (Hall et al. 2006). Perhaps a reconsideration of mandatory quarantine for domesticated pets should be reconsidered, as it not only prevented rabies from entering the Hawaiian ecosystem but reduced other pathogens that could potentially infect monk seals (Littnan et al. 2007). Knowing the diseases an individual monk seal has may also discourage other monk seal initiatives, especially translocation (Aguirre et al. 1999). According to Aguirre (2000), translocation of an infected animal with morbillivirus could not only be devastating to the recipient population, but to the population as a whole.

Future research could determine if there is a difference in infection rate between the main Hawaiian Islands and the Northwestern Hawaiian Islands, as it is possible for seals from one area to infect seals of the other area (Littnan et al. 2007). Previous research has noted that infectious diseases are suspected in the deaths of monk seals from the Northwestern Hawaiian Islands

(Banish and Gilmartin 1992) - Littnan et al. (2007) noted that several monk seals were individuals translocated from the Northwestern Hawaiian Islands to the main Hawaiian Islands.

A broader knowledge of diseases affecting Hawaiian monk seals may help describe the status of the population and possibly quantify how badly it has been affected. Mediterranean monk seals suffered a devastating epidemic of morbillivirus that is believed to be unrelated to toxins (Osterhaus et al. 1998), reducing the population of the 300 monk seals living on the African coast to less than 150 individuals (van de Bildt et al. 1999). The epizootic took a heavy toll on the adult population, which showed the most acute decline at the time with estimates that the adult population (those reproductively significant in the population) post-epizootic did not exceed 77 individuals and was probably smaller, illustrating the massive loss of genetic variability in the Mediterranean monk seal population (Forcada et al. 1999). Hawaiian monk seals may see a similar impact, as the population has thus far tested negative to morbillivirus (Aguirre et al. 2007).

Since it has been seen how damaging morbillivirus can be to an endangered population, preventative measures should be taken to protect the Hawaiian monk seal population from it and other diseases. However, the overall effect of other diseases on other marine mammal populations, except for the effects of morbillivirus on the Mediterranean striped dolphin population, has not been discussed in the literature. Age and sex determination of those seals that died of disease will show if certain age classes are more susceptible and if males or females are more susceptible. With these patterns, biologists and conservationists can conduct a pre-emptive strike on diseases by vaccinating those individuals that are more susceptible to infection.

The current author believes that a more thorough investigation of the diseases affecting monk seals will help quantify the effect they have on the population. Vaccination could in fact mitigate disease as a preventative measure, as suggested by Littnan et al. (2007) and Hall et al. (2006). No literature has been found on how vaccines would be developed and/or administered and what diseases would be vaccinated against. Given the potential effect of morbilliviruses (Hall et al. 2006, Littnan et al. 2007) and that toxoplasmosis has shown to be fatal in monk seals (Honnold et al. 2005), the author believes that preventative measures need to be taken and that vaccination protocols need to be developed. These protocols may even help the conservation and management of other marine mammal populations in Hawaii and around the world.

References

- Aguirre AA (2000) Health assessment and disease status studies of the Hawaiian monk seal (*Monachus schauinslandi*). Southwest Fisheries Science Center Administrative Report H-00-01: 44 pp.
- Aguirre AA, Keefe TJ, Reif JS, Kashinsky L, Yochem PK, Saliki JT, Stott JL, Goldstein T, Dubey TP, Braun R, and Antonelis G (2007) Infectious disease monitoring of the endangered Hawaiian monk seal. *J Wildl Dis* 43(2): 229-241
- Aguilar A and Raga JA (1993) The striped dolphin epizootic in the Mediterranean Sea. *Ambio* 22(8): 524-528
- Aguirre AA, Reif JS, and Antonelis GA (1999) Hawaiian monk seal epidemiology plan: health assessment and disease status studies. NOAA Technical Memorandum NMFS NOAA-TM-NMFS-SWFSC-280: 63 pp.

Banish LD and Gilmartin WG (1992) Pathological findings in the Hawaiian monk seal. *J Wildl Dis* 28(3): 428-434.

Forcada J, Hammond PS, Aguilar A (1999) Status of the Mediterranean monk seal *Monachus monachus* in the western Sahara and the implications of a mass mortality event. *Mar Ecol Prog Ser* 188: 249-261.

Gilmartin WG, Morrow RJ, and Houtman AM (1986) Hawaiian monk seal observations and captive maintenance project at Kure Atoll, 1981. NOAA Technical Memorandum NMFS NOAA-TM-NMFS-SWFC-59: 9 pp.

Hall AJ, Jepson PD, Goodman SJ, and Härkönen T (2006) Phocine distemper virus in the North and European seas- data and models, nature and nurture. *Biol Conserv* 131: 221-229.

Honnold SP, Braun R, Scott DP, Sreekumar C, and Dubey JP (2005) Toxoplasmosis in a Hawaiian monk seal (*Monachus schauinslandi*). *J Parasitol* 91(3): 695-697.

Littnan CL, Stewart BS, Yochem PK, and Braun R (2007) Survey for selected pathogens and evaluation of disease risk factors for endangered Hawaiian monk seals in the Hawaiian Islands. *EcoHealth* 3: 232-244.

Lu Y, Aguirre AA, Wang Y, Zeng L, Loh PC, and Yanagihara R (2003) Viral susceptibility of newly established cell lines from the Hawaiian monk seal *Monachus schauinslandi*. *Dis Aquat Org* 57: 183-191.

Nielsen O, Nielsen K, Braun R, and Kelly L (2005) A comparison of four serologic assays in screening for *Brucella* exposure in Hawaiian monk seals. *J Wildl Dis* 41(1): 126-133

Osterhaus A, Groen J, Niesters H, van de Bildt M, Martina B, Vedder L, Vos J, van Egmond H, Sidi BA, and Barham MEO (1997) Morbillivirus in monk seal mass mortality. *Nature* 388: 838-839

Osterhaus A, van de Bildt M, Vedder L, Martina B, Niesters H, Vos J, van Egmond H, Liem D, Baumann R, Androukaki E, Kotomatas S, Komnenou A, Sidi BA, Jiddou AB, and Barham MEO (1998) Monk seal mortality: virus or toxin? *Vaccine* 16(9/10): 979-981.

Osterhaus A, Visser IKG, de Swart RL, van Bresse MF, van de Bildt MWG, Örvell C, Barrett T and Raga JA (1992) Morbillivirus threat to Mediterranean monk seals? *Vet Rec* 130: 141-142

van de Bildt MWG, Martina BEE, Sidi BA, and Osterhaus ADME (2001) Morbillivirus infection in a bottlenosed dolphin and a Mediterranean monk seal from the Atlantic coast of West Africa. *Vet Rec* 148(7): 210-211.

van de Bildt MWG, Vedder EJ, Martina BEE, Sidi BA, Jiddou AB, Barham MEO, Androukaki E, Komnenou A, Niesters HGM, and Osterhaus ADME (1999). Morbilliviruses in Mediterranean monk seals. *Vet Microbiol* 69: 19-21

Yantis D, Moeller R, Braun R, Gardiner CH, Aguirre A, and Dubey JP (2003) Hepatitis associated with a *Sarcocystis canis*-like protozoan in a Hawaiian monk seal (*Monachus schauinslandi*). *J Parasitol* 89(6): 1258-1260.