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HAWAIIAN MONK SEAL EPIDEMIOLOGY PLAN: HEALTH ASSESSMENT AND DISEASE STATUS STUDIES

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U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
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EXECUTIVE SUMMARY

The Hawaiian monk seal (*Monachus schauinslandi*) is one of the most endangered marine mammals in the world. Populations of Hawaiian monk seals have shown a decline in recent years which has placed the species in threat of extinction. Understanding the potential role of disease and toxins is a high priority. Several natural sources of mortality have been identified or suggested (e.g., ciguatera poisoning, starvation, shark predation, trauma/mobbing, and disease), but the relative significance of these factors and their effect on population trends are poorly understood. Efforts to enhance the recovery of the Hawaiian monk seal will require a better understanding of the health and disease status of the wild population. Thus, health and disease impacts on the population merit a cohesive, well-supported effort to mitigate potential effects.

The Epidemiology Plan was developed to prioritize and implement projects regarding health and disease for the Hawaiian monk seal. As a fundamental component of the research and recovery activities conducted by the Marine Mammal Research Program (MMRP), Protected Species Investigation, National Marine Fisheries Service, Honolulu Laboratory, the Epidemiology Plan incorporates specific strategies under the broad heading of health and disease intended to enhance recovery and prevent further decline of the species. In developing this plan, consideration was given to priorities assigned to specific research tasks outlined in the *Recovery Plan for the Hawaiian Monk Seal, Monachus Schauinslandi* and the recommendations of the Hawaiian Monk Seal Recovery Team (HMSRT) at its annual meetings.

The eventual intent is to develop a long-term plan for addressing various health and disease projects that address management and recovery of the species. For example, disease surveillance, the health and disease aspects of translocation efforts, and the development of contingency plans for unusual mortality and exposure to anthropogenic contaminants, spills, biotoxins, or natural disasters are considered. A component of the plan includes ongoing, prospective health assessment of monk seal subpopulations to monitor temporal changes in health status and to determine the effect on population abundance and reproductive success.

The Epidemiology Plan is further intended to be a working document that will serve as a guide for biomedical research to standardize field procedures relevant to health and disease. The Plan is intended to be used by veterinarians, disease specialists, and epidemiologists.

The mission of the Epidemiology Plan is to provide a framework for incorporation of health and disease information to enhance recovery of the species. At the population level, the mission statement can be defined as a series of epidemiologic objectives. The epidemiology plan is a long-term strategic plan that addresses studies to assess health and disease prevalence in monk seal subpopulations, retrospective analyses of previously collected data, prospective studies of health and disease in monk seal subpopulations, contingency plans for unforeseen events, and the role of epidemiologic methods in translocation efforts and rehabilitation.

The principal objectives of the Epidemiology Plan are to (1) conduct a current health assessment of the Hawaiian monk seal population, requiring the definition of baseline values for health parameters; (2) determine and quantify the principal components of suboptimal health and disease that may be contributing to the decline of the species in each subpopulation; (3) conduct retrospective analyses of archived biological materials collected in previous years and incorporate results in the data base bank; (4) prospectively evaluate the health and disease status of each subpopulation in order to assess changes in health status over time and evaluate the effectiveness of any interventions that may be introduced or the impact of environmental changes which may occur; (5) develop prevention and control strategies to mitigate the effects of suboptimal health and disease to enhance recovery and to prevent the introduction of disease into monk seal populations; (6) minimize potential future impacts on the health of monk seals by development of appropriate contingency and response plans; (7) integrate health and disease activities into future plans for translocation; and (8) integrate health and disease activities into further efforts to rehabilitate Hawaiian monk seals.

INTRODUCTION

Mass mortalities of marine mammals from disease are well documented (Harwood and Hall, 1990). Such mortality may play an important role in the dynamics of pinniped populations, with important implications for the genetics and evolution of the species. As demonstrated in other endangered species, disease can be catastrophic for the population and constitutes the force leading to extinction. The consequences of disease may be also important at the immediate level of the individual and the population (Cunningham, 1996). Diseases can cause mortality, increased susceptibility to predation or further disease, lower reproductive potential, or a combination of any of these. A recent example of the effects of mass mortality occurred in the Mediterranean monk seal (*Monachus monachus*), one of the most endangered pinnipeds in the world. Two subpopulations of the species exist: one on the Mediterranean coast with about 500 seals and the other on the Mauritanian coast (Cap Blanc) with about 310 seals. This last subpopulation experienced a catastrophic die-off from May to mid July 1997. Following the recovery of 117 carcasses, it was estimated that more than half of this western Sahara population was eliminated (Costas and Lopez-Rodas, 1998). Although the cause of mortality has raised considerable debate among European scientists, two morbilliviruses (Osterhaus et al., 1997, 1998) and algal biotoxins (Hernandez et al., 1998) have been identified in the laboratory from tissues of necropsied seals and postulated as possible etiologies.

The Hawaiian monk seal (*Monachus schauinslandi*) is among the most endangered species on earth and ranks near the top among the endangered marine mammals in terms of the critical status of the population. The Hawaiian monk seal population has been declining since the first range-wide surveys in the late 1950s and will likely continue to decline as a result of high juvenile mortality and low reproductive recruitment occurring at French Frigate Shoals (FFS), site of the largest subpopulation. If this trend continues, it is conceivable that the Hawaiian monk seal could be on the verge of extinction. Understanding the potential role of disease and toxins in this recent decline is a high priority. Several natural sources of mortality have been identified or suggested (e.g., ciguatera poisoning, starvation, shark predation, trauma/mobbing, and disease), but the relative significance of these factors and their effect on population trends are poorly understood. The prevalence and impact of other disease conditions have been inadequately studied. Efforts to enhance the recovery of the Hawaiian monk seal will require a better understanding of the health and disease status of the wild population. Thus, health and disease impacts on the population are a major concern and merit a cohesive, well-supported effort to mitigate potential effects.

Disease impacts include survival of juvenile seals, impairment of reproductive success, impaired growth, and development of juvenile seals; increased morbidity leading to debilitation and predation; and direct effects on survival of all age classes. A number of new infectious disease agents have been identified in marine mammal populations worldwide and have led to mass mortality events; e.g., the 1987-88 epizootic in North Atlantic harbor seals following introduction of a new morbillivirus later identified as phocine distemper virus and the 1987-88 die-off of bottlenose dolphins along the east coast of the United States later shown to be caused

by a second morbillivirus. Epizootics resulting in mass mortality are not uncommon among pinnipeds and may play an important role in population dynamics (Harwood and Hall, 1990). For the Hawaiian monk seal, the survival of the species may be threatened by a disease outbreak. The impacts of major mortality events such as those described above as well as the morbidity and mortality caused by endemic diseases justify interventions aimed at reducing their potential impact.

The Hawaiian Monk Seal Epidemiology Plan was developed to prioritize and implement projects regarding health and disease for the Hawaiian monk seal. As a fundamental component of the research and recovery activities conducted by the Marine Mammal Research Program (MMRP), Protected Species Investigation (PSI), National Marine Fisheries Service, Honolulu Laboratory, the Epidemiology Plan incorporates specific strategies under the broad heading of health and disease intended to enhance recovery and prevent further decline of the species. In developing this plan, consideration was given to priorities assigned to specific research tasks outlined in the *Recovery Plan for the Hawaiian Monk Seal, Monachus Schauinslandi* (Gilmartin, 1983) and the recommendations of the Hawaiian Monk Seal Recovery Team (HMSRT) at their annual meetings (Gilmartin, 1993). The eventual intent is to develop a long-term plan for addressing various health and disease projects for the management and recovery of the species. For example, disease surveillance, the health and disease aspects of translocation efforts, and the development of contingency plans for unusual mortality and exposure to anthropogenic contaminants, spills, biotoxins or natural disasters are considered in the plan. A component of the plan includes ongoing, prospective health assessment of monk seal subpopulations to monitor temporal changes in health status and determine the effect on population abundance and reproductive success.

The Epidemiology Plan is also intended to be a working document that will serve as a guide for biomedical research to standardize field procedures; provide protocols for sample collection, handling and transport during field activities; refine and standardize the health and disease database; and thus provide a framework for future epidemiologic and medical research in the Hawaiian monk seal. The Epidemiology Plan is also intended to be used by attending field veterinarians, disease specialists, and epidemiologists. All studies will follow the guidelines established within this plan, and new biomedical or disease research not described herein will be reported in a written protocol and added to this plan. This working document will receive annual revisions and additions. Much of the work presented here is linked to MMRP activities assigned as high priority by the HMSRT.

MISSION STATEMENT

The mission of the Hawaiian Monk Seal Epidemiology Plan is to provide a framework for incorporation of health and disease information to enhance recovery of the species. At the population level, this mission statement can be defined as a series of epidemiologic objectives. The epidemiology plan is a long-term, strategic plan which includes a pilot study to assess disease prevalence in monk seal subpopulations, retrospective analyses of previously collected

data, prospective studies of health and disease in monk seal subpopulations, contingency plans for unforeseen events, and the role of epidemiologic methods in translocation efforts and rehabilitation.

GOALS AND OBJECTIVES

The goals and objectives of the Epidemiology Plan include eight separate areas of activity:

(1) Conduct a current health assessment of the Hawaiian monk seal population, requiring the definition of baseline values for health parameters.

(2) Determine and quantify the principal components of suboptimal health and disease which may be contributing to the decline of the species in each subpopulation.

(3) Conduct retrospective analyses of archived biological materials collected in previous years and incorporate results in the data base bank.

(4) Prospectively evaluate the health and disease status of each subpopulation in order to assess changes in health status over time and evaluate the effectiveness of any interventions which may be introduced or the impact of environmental changes which may occur.

(5) Develop prevention and control strategies to mitigate the effects of suboptimal health and disease to enhance recovery and to prevent the introduction of disease into monk seal populations.

(6) Minimize potential future impacts on the health of monk seals by development of appropriate contingency and response plans.

(7) Integrate health and disease activities into future plans for translocation.

(8) Integrate health and disease activities into further efforts to rehabilitate Hawaiian monk seals.

HISTORICAL IMPACT OF DISEASE IN THE HAWAIIAN MONK SEAL

Several sources of natural mortality have been described in Hawaiian monk seals including mobbing (Johanos et al., 1990; Hiruki et al., 1993a,b); starvation (primarily affecting young seals (Banish and Gilmartin, 1992)), predation by sharks (particularly tiger sharks

(*Galeocerdo cuvier*) and Galapagos sharks (*Carcharhynchus galapagoensis*) (Balazs and Whittow, 1979; Alcorn and Kam, 1986; Hiruki et al., 1993a)), net entanglement (Henderson, 1990), and disease and trauma (Banish and Gilmartin, 1992; Hiruki et al., 1993a). The importance of endoparasites as a source of mortality is unknown although practically all seals carry them (Dailey et al., 1988). Biotoxins, including ciguatoxin and mitotoxin have been suspected as causes of mortality (Gilmartin et al., 1980).

Although some information concerning medical conditions affecting the Hawaiian monk seal is available, the etiology and impact of disease on wild animals at the population level is far from clear. There are substantial data gaps regarding the prevalence of disease conditions in populations of Hawaiian monk seals in the wild, and thus their potential impact on population dynamics is indeterminate. In the wild, even massive epizootics in remote locations may pass undetected. To date, there is no satisfactory observational way of determining whether a disease is impacting the population (McCallum, 1994). For example, the Hawaiian monk seal experienced a die-off in 1978 at Laysan Island (Johnson and Johnson, 1981). More than 50 seal carcasses were found in an advanced state of decomposition, and the cause of the mortality was not identified. Survival of immature seals severely declined at FFS after 1987, and the reproductive potential of the species was being seriously compromised with the loss of young females. The cause has been attributed to emaciation/starvation; however, the role of endoparasites or infectious disease remains unknown. During 1992-93, undersized pup and juvenile seals from FFS were rehabilitated and released at Midway Atoll with poor success. To date, the question remains whether *Salmonella* infections were attributed to the poor survival at Midway. In addition, the role of ciguatera poisoning in monk seal mortality remains unresolved. The most recent experience regarding disease investigation includes the eye condition diagnosed in 10 of 12 female seal pups brought to Oahu for rehabilitation in 1995. This blinding condition was characterized by blepharoconjunctivitis, photophobia, corneal opacities, and eventual cataract formation. To date, the etiology of that disease remains unknown despite intensive diagnostic investigation.

Attempts to document health and disease in Hawaiian monk seals began following the 1978 die-off at Laysan Island. Prior to that, some parasitology studies were conducted. Causes of mortality in the population were identified based on field necropsies of dead individuals and in some cases, on follow-up histopathologic studies. The primary causes of monk seal mortality from 1981 to1995, based on gross and histopathologic examination of 65 monk seals were identified as emaciation in 36 seals (35%), trauma in 19 seals (29%), infectious disease in 10 seals (15%), and undetermined mortality in 8 seals (12%) (Banish and Gilmartin, 1991; T. Work, 1996 unpubl. data). Gastrointestinal parasite burdens were abnormally high in 45 (69%) seals, but their significance remains unknown. The chronologic events related to health and disease investigations in the Hawaiian monk seal are summarized in Table 1.

The relative significance of these factors and their effect on population trends, in particular the role of disease, are poorly understood. Disease processes may be important determinants of population trends through long-term low levels of mortality, or through episodic die-offs. For example, the mass mortality of monk seals that occurred at Laysan Island may have been due to a disease process. Similarly, disease may be contributing to the high juvenile

mortality occurring at FFS since 1988. In addition, the potential for disease transmission has not been an important concern in management activities involving translocation of seals between reproductive sites to date. The role that disease may have played in the extensive loss of seals translocated to Midway from FFS in 1992-93 is unknown.

Table 1. Chronologic events of health and disease studies in Hawaiian monk seals (*Monachus schauinslandi*), 1925-97.

1925	Internal parasites were first reported (Chapin, 1925)
1952	Diphyllobothriid cestodes were first reported (Markowski, 1952)
1959	The Acanthocephalan *Corynosoma* sp.was first reported (Golvan, 1959)
1969	Diphyllobothriid cestodes were reported (Rausch, 1969)
1978	Known as the Laysan epizootic, $\geq$50 monk seals were found dead. Specimens from 19 dead and 18 live seals were collected. All carcasses found with stomach ulceration and heavy parasite burdens and in severe state of emaciation. Livers from two carcasses tested positive to ciguatoxin and maitotoxin. There was serologic evidence to caliciviruses but serum specimens were negative for *Leptospira. Salmonella sieburg* was isolated from a rectal swab. Many parasite ova and products in coprologic exams were identified. Diagnosis was inconclusive (Johnson and Johnson, 1981; Gilmartin et al., 1980).
1979	*Contracecum* ulceration of a young seal was first reported (Whittow et al., 1979)
1980	Lung mites from the family Halarechnidae were first reported (Furman and Dailey, 1980)
1980	The Hawaiian monk seal die-off response plan was developed with the support of the Marine Mammal Commission (Gilmartin, 1987)
1983	The Recovery Plan for the Hawaiian monk seal addressed the importance of disease investigations (Gilmartin, 1983)
1988	A coprologic survey for parasites was performed from field scats collected in 1985 (Dailey et al., 1988)
1988	The hematology and serum biochemistry of 12 weaned pups collected between 1984 and 1987 for their rehabilitation in Oahu were reported (Banish and Gilmartin, 1988)
1992	Pathology of 42 seals collected between 1981-85 was summarized (Banish and Gilmartin, 1992)
1992	The French Frigate Shoals relocation project of 19 immature seals was initiated. Basic hematology, serum biochemistry, serology for leptospirosis and calicivirus infection, virus isolation, fecal culture for *Salmonella* and coproparasitoscopic examination were performed for their disease evaluation. Two of seven seals died of bacterial and aspiration pneumonia in Oahu, with positive titers to *Leptospira*. Detection of calicivirus by cDNA hybridization probe in 13 seals with viral particles seen by electron microscopy occurred in five seals. It was concluded that endemic disease agents identified in those seals were *Salmonella* and endoparasites (Gilmartin, 1993a; Poet et al., 1993).
1993	Inoculation of four monk seals with a killed virus distemper vaccine was experimentally performed on three seals at the Waikiki Aquarium (Gilmartin, 1993b; Osterhaus, unpubl. data 1997).
1995	An eye disease of unknown etiology was first diagnosed in 12 female monk seal pups that were transported to Oahu for rehabilitation. To date the cause remains unknown (NMFS files 1995-97, unpubl. data).
1996	Histopathology of selected tissues collected from 23 seals between 1989 and 1995 was performed by personnel of the National Wildlife Health Research Center, Honolulu Station (T. Work, unpubl. data, 1996).
1997	Two captive seals died of causes unrelated to the eye disease. One seal was diagnosed with *Clostridium* septicemia and another seal with hepatic sarcocystosis (Yantis et al., 1998).
1997	The Monk Seal Captive Care Review Panel developed recommendations to evaluate the health assessment and future disposition of 10 captive seals and the future of captive care and release efforts to enhance the recovery of the species (NMFS, unpubl. data, 1997).

THE RECOVERY PLAN

Specific tasks regarding disease investigations are outlined in the *Recovery Plan for the Hawaiian Monk Seal, Monachus Schauinslandi* Sections 1.1; 1.4; 1.5; 5.23; 5.26; 5.27; 5.29. The first objective listed is to identify and mitigate, where possible, those natural factors causing or contributing to decreased survival and productivity. Specifically, the plan identifies the need to develop baseline data on diseases by performing necropsies, histopathologic examination, the plan identifies the need for determining parasite types and load, culturing bacterial and viral agents, and collecting tissue samples for toxicology. The study of sick seals includes documentation of signs of disease, collection of specimens, serologic testing, and treatment. Rehabilitation and release programs also require a thorough understanding of disease processes in order to address the feasibility and advisability of returning rehabilitated seals to the wild.

Assessment of the relationship between monk seals and biotoxins such as ciguatera is also addressed by the Recovery Plan. It specifically describes the need to assess and monitor ciguatera levels in prey food items, assess biotoxicity in monk seals and develop treatment methods as necessary. The development of these baseline data includes the implementation of standardized procedures and forms. Other recommended actions within the Recovery Plan include the completion of a mass mortality reaction plan (Gilmartin, 1987), the development of a response plan for oil and chemical spills, and interpretation of results to initiate appropriate management actions.

Recovery work plans (Gilmartin, 1990; 1993a; 1993b; HMSRT, 1995; Captive Care Review Panel, 1997) outline the significant accomplishments and events related to monitoring and recovery of the species. Activities that pertain to Hawaiian monk seal health and disease investigations include the following:

(1) Reintroduction of rehabilitated females to Midway Islands in 1992, but no further releases occurred at this site because of observed high mortality.

(2) Collection of underweight weaned female pups at FFS for rehabilitation and reintroduction at Kure Atoll.

(3) Infectious disease monitoring at FFS concluded that disease was not a factor in the catastrophic decline.

(4) Cessation of rehabilitation efforts in 1995 due to an eye disease of unknown etiology.

(5) A Captive Care Review Panel was convened in 1997 to provide recommendations on the disposition of captive seals and future translocation efforts.

These findings emphasize the importance of disease screening when considering

rehabilitation and translocation of weaned pups to enhance juvenile survival. Additionally, future plans for translocation must include developing a plan in response to an epidemic, gathering historical information on infectious agents in other pinniped populations; assessing the potential of spreading diseases to other subpopulations; evaluating the probability of treatment or immunization; and determining the feasibility of implementing programs to control or treat seals diagnosed with a specific pathogen.

OBJECTIVE 1: CHARACTERIZE BASELINE HEALTH PARAMETERS FOR THE HAWAIIAN MONK SEAL

The first step in conducting a current health assessment of the population is to characterize the baseline health parameters for the 'normal' Hawaiian monk seal. In order to meet this objective, it is necessary to establish normal values for the Hawaiian monk seal based on 1) existing archived data, 2) data from processing of stored, unanalyzed samples where appropriate, 3) opportunistic collections of material from healthy animals, and 4) targeted collections of appropriate materials from clinically healthy Hawaiian monk seals.

Additional work to be done under this objective includes 1) development of testing protocols as required, 2) identification of suitable diagnostic procedures and tests, 3) identification of laboratory support, 4) development of a quality control/assurance plan for laboratory results, and 5) development of data management and data analysis plans. In general, baseline parameters which need to be established quantitatively or further refined include the following:

(1) Clinical Exam and Morphometric Measurements
(2) Hematologic and Biochemistry Evaluation
(3) Serologic profile for antibodies to infectious agents
(4) Virology Profile
(5) Bacteriology Profile
(6) Parasitology Profile
(7) Endocrinology Profile (for evaluation of growth and reproduction)
(8) Immunology Profile
(9) Toxicology Profile
(10) Biotoxicology Profile
(11) Pathologic Evaluation

Development of Diagnostic Testing and Other Protocols for Baseline Health Evaluation

Protocols have been developed to provide a systematic documentation of events and data collection to establish appropriate procedures in the field and laboratory. They also provide consistent techniques for the collection and evaluation of biomedical specimens and data. Existing protocols have been revised and updated, and new protocols should be designed and implemented as needed. To date, the following protocols have been revised, developed, or

included in the MMRP *1998 Field Manual for Research on the Hawaiian Monk Seal*:

(1) Chemical Immobilization
(2) Emergency Care
(3) Eye Examination
(4) Hematology & Biochemistry
(5) Microbiology (virology & bacteriology)
(6) Necropsy
(7) Parasitology
(8) Physical Examination
(9) Specimen Collection
(10) Tissue/Serum Banking

The following additional protocols need to be developed:

(1) Clinical Laboratory Techniques
(2) Data Base
(3) Diagnostic Procedures
(4) Laboratory Quality Control
(5) Medical Record Keeping
(6) Preventive Medicine
(7) Quarantine
(8) Regulatory Compliance
(9) Surveillance
(10) Translocation/Reintroduction
(11) Other

Identification of Diagnostic Procedures and Tests

Diagnostic tests and procedures that are incorporated into the protocols for health assessment activities, translocation, and rehabilitation projects are described below.

Chemical Immobilization

The development of improved methods for chemical restraint of wild monk seals that minimize stress during handling are required during sampling for health assessment. The *Chemical Immobilization Protocol for Hawaiian Monk Seals* (*1998 Field Manual for Research on the Hawaiian Monk Seal*) summarizes recommended dosages for use of Diazepam as a sedative and details the legal regulations for its use. Diazepam is an effective pharmaceutic for handling wild Hawaiian monk seals. However, its intravenous administration requires several people to manually restrain a seal.

Clinical Exams

When conducting a clinical exam the attending veterinarian will collect data on the age, sex, length, and axillary girth of all seals examined. The essential elements will be to detect clinically apparent signs of illness or trauma, evaluate body condition, and determine reproductive status. Special attention will be devoted to clinical findings that might indicate an infectious disease process: fever, ocular or nasal discharge, cough, labored respiration, inappetence, diarrhea, and dermal lesions. The *Hawaiian Monk Seal Clinical Examination Protocol* has been developed and incorporated into the *1998 Field Manual for Research on the Hawaiian Monk Seal*.

Clinical Pathology

Evaluation of hematologic and biochemical parameters is essential in health screening. A blood sample is required for completion of this requirement and for serological screening. At minimum, a standard screen includes a complete blood count (CBC) and differential count, total protein, albumin, globulin, total bilirubin, direct bilirubin, alanine aminotransferase (ALT or SGPT), aspartate aminotransferase (AST or SGOT), alkaline phosphatase (AP), creatinine kinase (CK), gamma glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), BUN, creatinine, calcium, phosphorus, bicarbonate, cholesterol, triglycerides, glucose, iron, sodium, potassium, chloride, A/G ratio, B/C ratio, Na/K ratio and anion gap (Aguirre, 1998). The available hematological values for wild Hawaiian monk seals are based on seals captured and released for rehabilitation purposes between 1989-94. Hematological and biochemical analyses of blood represent an important component for assessing health and disease status in the Hawaiian monk seal population. Research will be focused on quantitative techniques for evaluating health status and body condition using blood parameters based on other pinniped species (Rea et al., 1998).

Serology

Serological evaluation of antibody profiles to viral and bacterial pathogens is a primary objective of disease screening and baseline monitoring. Serologic testing (measurement of serum antibody) is the most frequently used technique in wildlife disease surveys. A number of factors; however, such as size and distribution of the samples, titer and duration of infection, behavior, and host longevity, may bias the information obtained from these surveys. Two qualitative errors will be considered in evaluating the significance of serology results: 1) samples from infected animals may be classified as negative (false negatives), and 2) samples from animals never exposed to the disease may be categorized as positive (false positives).

Despite these limiting factors, serologic surveys have many advantages. Blood samples are relatively easy to collect, periodic samples can be collected from the same animals over time providing information on exposure, laboratory tests are relatively inexpensive to perform, and a single sample can be tested for evidence of different disease agents. Properly stored sera are stable for long periods of time, providing a bank of information that can be tested in the future as new tests become available. If sample size is adequate, it is possible to evaluate the disease status of a population, and if populations are monitored over time, changes in the disease status can be determined. Early warning in changes of the disease status in a population can provide us with timely information so that appropriate disease control or management techniques can be applied if necessary (Zarnke, 1986). Serologic testing for the agents described in Table 2 will be

incorporated into the initial health assessment (Objective 2), retrospective studies (Objective 3), prospective studies (Objective 4) and translocation activities (Objective 7).

Serologic screening for infectious agents is highly preferable to attempts at isolation and culture. Seropositive animals may no longer be shedding the organism and may no longer be infective; however, given the serious consequences of introduction of any of these agents into a naive population, the implications of positive serology may be adequate to recommend against translocation.

Table 2. Diagnostic and laboratory tests of sera from wild Hawaiian monk seals (*Monachus schauinslandi*) to assess presence of selected infectious agents.

Diagnostic laboratory	Disease agent	Test procedure (positive titer)
IDDEX CVD, Sacramento, CA	*Dirofilaria immitis*	ELISA
Oklahoma Animal Disease	*Brucella abortus*	Card
Diagnostic Lab, Stillwater, OK	*Brucella canis*	Card
	Canine distemper virus	Serum neutralization
	Dolphin morbillivirus	Serum neutralization
	Phocine distemper virus	Serum neutralization
	Porpoise morbillivirus	Serum neutralization
	Phocine herpes virus 1	Virus neutralization
Oregon State University	*Leptospira* spp. -8 serovars	Microscopic agglutination
Callicivirus Research Laboratory,	Caliciviruses (39 serotypes)	Serum neutralization
Corvallis, OR	California sea lion rotavirus	Serum neutralization
	Fur seal herpesvirus	Serum neutralization
	Walrus adenovirus	Serum neutralization
	Walrus enterovirus	Serum neutralization
	Walrus retrovirus	Serum neutralization
USDA Foreign Animal Disease	Seal influenza virus	Reference antisera H & N
Diagnostic Lab, Plum Island, NY	Caliciviruses (17 serotypes)	Serum neutralization
USDA National Veterinary	*Brucella* spp.	BAPA, card, CF & rivanol
Services Labs, Ames, IA	*Chlamydia psittaci*	Complement fixation
USDA Zoonotic Disease Lab	*Toxoplasma gondii*	Modified agglutination
Beltsville, MD		

A potential weakness of using serologic screening to identify suitable candidates for translocation is the lag time between initial infection and the development of circulating antibody titers capable of detection in the laboratory. This period is generally between 2 and 4 weeks for most infectious agents, typically closer to 2 weeks. As an additional safeguard against this unlikely possibility, clinical observation of animals held for translocation following blood sampling disclose early signs of illness (incubationary carriers) and is a standard element of quarantine protocols. Antibody titers against some infectious agents may wane with time, leading to the possibility of recrudescence of a latent infection in a serologically negative animal (latent carriers). This situation may be encountered with some species of herpes viruses. Under stress, viral shedding may recur, usually with concurrent expression of clinical illness. In this event,

monitoring for clinical signs in conjunction with serologic screening affords the best protection against introduction of a pathogenic agent.

Identification of infectious agents, particularly viruses, by culture and isolation is less desirable than serologic screening for several reasons: 1) the agents are relatively fragile and may be destroyed or inactivated by improper handling or storage conditions; 2) the agents may be detectable for only a brief period during the infectious process, at the height of viremia or bacteremia; 3) isolation depends on the availability of suitable substrates such as cell lines which support the growth of the agent; 4) isolation and culture techniques are generally more expensive on a per sample basis; 5) isolation techniques are generally less sensitive than serologic screening; and 6) the time required for isolation and identification of viruses generally exceeds that required for serologic screening. The exception to this general rule is the isolation of enterobacteria such as *Salmonella* by direct culture.

Serologic tests such as those described below generally identify IgG antibodies. Serum IgG antibody concentrations may result from previous infection, hence their usefulness in population screening. Following initial infection, the first antibody response is comprised of IgM proteins. This is followed by the production of IgG antibodies which tend to persist for longer periods of time (years for many viral infections such as morbillivirus). Therefore, IgG antibody does not necessarily indicate an active infection with the agent. IgG antibody may also be transferred passively from mother to fetus through the placenta or in colostrum through nursing.

Virology

Buffy coats obtained from the blood, external lesions/blisters, and natural orifice swabs, in addition to tissues from necropsied seals, will be submitted for virus isolation. Recently, an acute eye disease characterized by corneal opacities and bilateral cataracts has developed in captive Hawaiian monk seals. Studies have suggested a possible association between phocine herpesvirus and this eye disease. However, no virus has been isolated and identified from the diseased animals (Aguirre et al., 1998). The development of a standard immunological-based diagnostic assay is underway for the early detection of viral infections in monk seals by using polyclonal antibodies to be produced against monk seal immunoglobulins (IgG). Several diagnostic laboratories have requested the availability of anti-monk seal IgG. Cell lines from various tissues/organs of Hawaiian monk seals have been established (Lu et al., 1998). Because of the potential importance of infectious diseases in populations of Hawaiian monk seals, the available information concerning the major viral diseases of potential significance for objectives 2 through 8 is briefly summarized in Appendix II.

Bacteriology

Routine microbiological evaluation of respiratory, gastrointestinal, and ocular flora will be established to provide an understanding of normal patterns. Rectal swabs will be collected for isolation and characterization of *Salmonella* spp. and other enterobacteria. *Salmonella* has been implicated as a mortality factor and has been isolated in many of the seals brought for rehabilitation. In addition, it has been speculated that this organism may be endemic to monk

seals. Further research to address some of these questions is underway. Because of the potential importance of infectious diseases in populations of Hawaiian monk seals, the available information concerning the major bacterial diseases of potential significance for objectives 2 through 8 is summarized in Appendix II.

Parasitology

Moderate to severe parasitic gastric ulcerations have been observed in almost all postmortem examinations performed on Hawaiian monk seals. Parasitism was reported as a pathological finding in 37 of the 42 monk seals necropsied by Banish and Gilmartin (1992) from 1981 to 1985. Four species of cestodes, two nematodes, one acanthocephalan, and trematodes were reported in this survey. Of potential significance was the finding of gastric ulcers with attached *Contrcaecum* parasites and the report of a similar finding during an investigation of monk seal mortality on Laysan Island (Gilmartin et al., 1980). The role of parasitism in frequently reported emaciated monk seals or causing debility adequate to increase susceptibility to seals by predators such as sharks is unknown. Cestode parasites (tapeworms) may be minimally pathogenic unless they exist in adequate numbers to cause a mechanical bowel obstruction or compromise nutrient absorption. Trematodes are most frequently found in liver where they cause lesions such as bile duct obstruction. Infestations with Acanthocephalans (thorny-headed worms of the genus *Corynosoma),* and nematodes (roundworms) of the genus *Contracecum* are harmful and likely to impair health. These parasites are pathogenic by virtue of their ability to cause inflammatory lesions, gastric ulceration, altered gastrointestinal physiology, decreased nutrient absorption, blood loss, and anemia. Most of the parasite material has not been systematically accessed or archivally preserved, and little of it has been studied. The detailed description, evaluation and recording of all such biological specimens is the first step in determining research priorities and designing prospective studies. Tissue and parasite specimens collected from monk seals since 1989 are currently being analyzed for taxonomic and pathologic studies. Other studies will include life cycles, diversity and burdens, pathologic impact and biology of prevalent parasite species. In FY98 parasite studies were limited to helminth parasites and related organs, tissues, spews, and fecal materials archived at the Honolulu Laboratory.

Endocrinology

The endocrinology of Hawaiian monk seals is relatively unknown. Limited reproductive research for captive seals has indicated that Hawaiian monk seals are seasonal polyestrous breeders. Males' seasonal testosterone levels peak in early summer (Atkinson, 1997). Further studies must include specimens from the same individual in a population. A selected hormonal panel will be tested to establish baseline data for the species in the wild. Emphasis will be given to growth, stress, and reproductive hormones. Development of an endocrinologic profile for the monk seal may also be useful in understanding the phenomenon known as "male mobbing" which has had an impact on monk seal populations.

Immunology

The development and application of immunologic techniques for the Hawaiian monk seal is recommended. Evaluation of immune function is a potentially useful tool for health assessment. Direct applications of immune testing include: 1) assessment of effects of accumulation of contaminants such as PCBs known to cause immunosuppression in other species. Currently the impact of burdens of contaminants on health for the monk seal is unknown, 2) assessment of immune function for resistance to infectious agents, 3) assessment of effects of translocation or rehabilitation on immune function and evaluation of alternative strategies such as hard vs. soft release, and 4) assessment of general environmental conditions on immune function; e.g., stress from male mobbing. Tests for immune function will be developed for the monk seal as well as for other marine mammals. A battery of tests capable of evaluating the function of both humoral and cell-mediated responses is needed. Examples of tests which may be useful include humoral response to foreign proteins such as killed infectious agents, functional assessment of lymphocyte responsiveness to mitogens, production of cytokines such as the interleukins, and differential counts of lymphocyte subpopulations (i.e., cd4:cd8 ratios, NK cells).

Toxicology

Toxicological studies will be conducted to determine if these compounds, which are known to occur at extremely high concentrations in other species of marine mammals, play a significant role in the health of wild Hawaiian monk seals. The evaluation of residues of potentially important contributors to health status will be based on the collection of blubber biopsies and blood from live seals and from selected tissues (blubber, liver, kidney, brain, stomach contents) in necropsied seals. All tissues will be analyzed for chlorinated hydrocarbons (CHs) and toxic metals (mercury, selenium, aluminum, copper, zinc, arsenic, cadmium, lead, nickel) by a screening method using high performance liquid chromatography couple with photodiode array detection developed by NMFS Environmental Conservation Division (ECD), Seattle, WA (Khran et al., 1994). The CH analytes included are selected polychlorinated biphenyls (PCBs) congeners, DDTs and their metabolites (DDEs, DDDs), and hexachlorobenzene (HCB). These analytes are potentially toxic, persist in the environment and are bioaccumulated by marine mammals. Selected samples will be analyzed by gas chromatography/mass spectrophotometry to provide a more complete description of the contaminants present in blood and blubber (Khran et al., 1997). Tissues (liver, kidney, blubber) from necropsied seals will also be collected following strict protocols for the National Biomonitoring Specimen Bank, National Institute of Standards and Technology, Charleston, South Carolina, to provide a resource that can be used for future retrospective analyses and documentation of long-term trends in environmental quality. The results from this analysis will provide baseline information on chemical contaminants, biochemical components, biotoxins, define guidelines for minimizing tissue sample variability, and ensure accuracy, precision, level of detection, and intercomparability of data resulting from chemical analyses of tissue samples (Becker et al., 1997).

Biotoxicology

The HMSRT recommended in 1989 that monk seal tissues and monk seal prey be surveyed for ciguatoxin at Midway prior to translocation efforts of 1992. Ciguatera poisoning was postulated as a possible cause of the 1978 Laysan Island monk seal mortality (Gilmartin et al., 1980), and historical outbreaks of ciguatoxic fish have been reported at Midway (Wilson and Jokiel, 1986). Ciguatera fish poisoning is caused by the ingestion of a wide variety of coral reef fishes that contain toxins accumulated through the food web. Ciguatoxin is a polyether compound produced by the benthic dinoflagellate *Gambierdiscus toxicus* associated with microalgae. When ingested, ciguatoxin accumulates in liver, gonads, and muscle. It is hypothesized that dinoflagellate blooms may be responsible for some intermittent monk seal mortality. Ciguatera fish poisoning has characteristic gastrointestinal and neurologic signs and is occasionally fatal (Withers, 1982). Ciguatoxic fish have been identified in areas of human disturbance; i.e., dredging (Lewis, 1984). Immunohistochemistry, mouse bioassays, receptor assays, and mass spectrophotometry will be investigated as possible techniques to detect ciguatoxin in reef fish and seal tissues collected by field personnel. This testing may provide baseline patterns in wild Hawaiian monk seals and ciguatoxic areas during translocation efforts.

Pathologic Evaluation of Monk Seal Tissues

Continuing pathologic examination of available monk seal tissues is strongly recommended for documenting causes of mortality in the population. This activity is required to validate the significance of clinical procedures that are used for screening and health assessment and to make definitive diagnoses of causes of death for incidental mortality, episodic mortality, and mortality that may accompany translocation or rehabilitation efforts. The availability of continuing support to the program from a board-certified veterinary pathologist is recommended in order to accomplish this task.

Identification of Laboratory Support

Diagnostic laboratory testing plays an important role in the monitoring of health status of Hawaiian monk seals. During these studies, tests will be used to detect exposure to an agent (serology) and to detect and identify the agent involved in an individual animal infection (bacteriology/virology) or an unusual mortality event (clinical pathology, gross pathology, histopathology). Tests will be used for epidemiologic purposes including estimation of prevalence, incidence, and geographic distribution of selected disease agents; determination of infection in the subpopulations; determination of disease risk factors including intraspecific and interspecific transmission (Gardner et al., 1996).

Selection of laboratories to test specimens of Hawaiian monk seals will be based on recommendations of the Clinical Laboratory Improvement Act 42 CFR 493 (Clinical Laboratories) and 21 CFR 58 (Good Laboratory Practices), the National Committee for Clinical Laboratory Standards, the American Association of Veterinary Laboratory Diagnosticians, and the NMFS Marine Mammal Health and Stranding Response Program.

The testing laboratory must be able to demonstrate that it is capable of performing the tests

required. The laboratory must operate an internal quality assurance program appropriate to the type, range, and volume of work performed. Standard Operating Procedures (SOP) must be documented in a manual and be available for use by laboratory staff. This manual must be maintained as relevant and current by the testing laboratory's quality assurance officer. The manual will include organizational charts (structure of laboratory), operational and functional duties, general quality assurance procedures and quality control procedures specific for each test, proficiency testing, use of reference sera or controls, and a procedure to deal with technical problems.

The staff will be trained, with technical knowledge and necessary education for the assigned functions. Training records regarding qualification procedures of personnel must be documented and personnel and tests must be validated at least 3 times. All equipment will be properly maintained to ensure protection from corrosion and other causes of deterioration, and all measuring and testing equipment will be calibrated as appropriate. A calibration schedule will also be maintained. Reference standards held by the laboratory will be used for calibration of equipment that can provide traceability to a national or international standard of measurement.

Development of a Quality Control/Assurance Plan

The testing laboratory must have adequate documented instructions on the use and operation of all relevant equipment, on the handling and preparation of test specimens, and on standard testing techniques. Also, it must have Standard Analytical Methods (SAM) and procedures required by the specification against which the specimens are to be tested. Non-standardized procedures will be fully documented. The environment in which the tests are undertaken must not invalidate results or adversely affect accuracy and precision. The testing facility must be protected from excessive temperature, dust, moisture, steam, vibration, electromagnetic disturbance, and interference. Chain of custody and a system for identifying the samples are necessary to assure that there will be no confusion regarding the identity of the samples and the results of measurements made. At all stages of storing, handling, and preparation of specimens, precautions must be taken to prevent any damage to the specimens that would invalidate the results. There should be clear rules for receipt, retention, and disposal of specimens.

The testing laboratory will maintain a record system and comply with existing regulations, and records will be maintained from original observations, calculations of derived data, calibration records, and the final test report. The records must contain sufficient information to permit repeatability of the test. All records and test results must be held secure and in confidence. A test report will be prepared by the testing laboratory clearly and unambiguously presenting the test results and relevant information. The testing laboratory must have a procedure for a fail test with a backup procedure to determine what will be done in the case of testing failure. A plan will be in place for expiration of reagents and chemicals, and daily log books must be maintained for temperature controls and incubators. Periodically, NMFS will audit the testing laboratory to document good laboratory practices.

Development of a Data Management and Data Analysis Plan

The data base developed by the Epidemiology Plan will be linked to the general data base of MMRP. Currently, the general data base includes information on transport, daily care records, hematology/serum biochemistry, and survival factors reported in captive and free-ranging monk seals. In addition, the Epidemiology Plan data base will include information regarding clinical and pathological records, the standard reference collection (normal physiological data), pharmaceutical records, diagnostic test results, and disease diagnosis describing causes of morbidity and mortality of captive and free-ranging monk seals.

Biological specimens from live and necropsied monk seals will be archived and actively managed. Detailed descriptions, evaluations, and records of these biological specimens are the first steps to determine research priorities and design of retrospective studies. Specimens will be archived by diagnostic activity, type of specimen, preservation medium, and laboratory test performed.

Centralized management of all biological specimens including the permanent serum bank and records/archives of all Hawaiian monk seals within the agency and in possession of other investigators has been one of the primary tasks initiated by the Epidemiology Program. The acquisition of a new ultra freezer and the remodeling of the Diamond Head Laboratory at Kewalo Research Facility (KRF) have facilitated the centralization and management of specimens. Specimens from monk seals are located in a variety of institutions at present. These materials will be collected and stored in a central location at the Honolulu Laboratory for disposition, further study, or discard.

OBJECTIVE 2: CONDUCT BASELINE HEALTH ASSESSMENT OF THE HAWAIIAN MONK SEAL

This objective requires the performance of a health assessment for the six reproductive subpopulations of Hawaiian monk seals at FFS, Midway Atoll, Laysan, Lisianski, Pearl and Hermes Reef, and Kure Atoll. The purpose of the health assessment is to establish the current health status of each of the subpopulations. The information will be used to 1) target any impacted subpopulations for additional investigation of specific diseases that may be identified, 2) develop plans for mitigation or rehabilitation if indicated and feasible, and 3) identify suitable subpopulations for translocation efforts.

- Develop a sampling plan for each subpopulation to include sites, numbers of animals to be sampled, and age and gender groups.

Work under this objective is in progress. A pilot study was conducted during 1998 in which baseline parameters were measured on 107 monk seals; 51 from FFS, 10 from Midway and 46 from Pearl and Hermes Reef. The objectives of the pilot study were 1) to assess baseline biomedical information from three reproductive populations of monk seals; 2) to assess evidence

of exposure to specific infectious disease agents in the three sampled populations using standard diagnostic procedures for serology, bacteriology, virology, and parasitology; 3) to assess body burdens of anthropogenic contaminants using standard toxicological procedures; and 4) to evaluate the results obtained and provide recommendations regarding translocation strategies involving the three sampled subpopulations.

The sampling plan for future health assessment and disease status studies will consider the six subpopulations including seals of all age cohorts and both sexes, with the exception of obviously pregnant seals and mothers with pups. Forty seals will be sampled at each subpopulation to detect antibodies to selected infectious disease agents and to determine prevalence of infection on a given subpopulation (see Objective 7 Section 11 and Table 3). It is recommended that, with the exception of the need to resample the Midway Atoll population in 1999, each subpopulation be resampled every other year.

- Select and conduct diagnostic tests in each subpopulation

The selection of health parameters and infectious agents are in the list of previously established procedures outlined under objective 1.

- Develop and maintain a data base for storage of information from this activity

- Conduct statistical evaluation of the data by age class and subpopulation

OBJECTIVE 3: CONDUCT RETROSPECTIVE HEALTH ASSESSMENT OF THE HAWAIIAN MONK SEAL

This objective will utilize the collection, cataloging, and analysis of Hawaiian monk seal samples stored at KRF and elsewhere. These materials have been collected over time and represent a resource to enhance our understanding of baseline health parameters for the monk seal. The need for a better understanding of health and disease has long been recognized, and the Epidemiology Task will build on preliminary work and consolidate the information generated by field personnel since 1984. The following steps are being taken regarding these specimens:

(1) An inventory of available materials which comprise tissues, sera, and results of hematological and biochemical analyses.

(2) Development of a computerized database to permit the tracking and identification of historical samples with respect to their origins from specific Hawaiian monk seals subpopulations over time. The materials are presently located in a variety of institutions. We have attempted to collect and store these specimens in a central location at the Honolulu Laboratory for disposition, further study, or discard.

(3) Prioritization of analyses for specific samples will begin to maximize the

usefulness of the data and fill critical data gaps. The primary purposes of this analysis will be to develop tools for health assessment of wild Hawaiian monk seal subpopulations and to aid in developing management strategies for recovery including translocation and rehabilitation. The additional analyses required will include serological evaluation for antibodies to selected viral and bacterial agents; immunochemistry for infectious agents and biotoxins; histopathologic evaluation of stored tissue for general health status and immune status; and identifying, mounting, and developing a data base of stored parasites collected from Hawaiian monk seals since 1989.

OBJECTIVE 4: CONDUCT PROSPECTIVE HEALTH ASSESSMENT OF THE HAWAIIAN MONK SEAL

This objective requires continuation of ongoing prospective health assessments for each subpopulation of Hawaiian monk seals at FFS, Midway Atoll, Laysan, Lisianski, Pearl and Hermes Reef, and Kure Atoll. The purpose of the health assessment is to 1) monitor temporal changes which may be occurring in health status and determine the effect on population abundance and reproductive success, 2) evaluate the effectiveness of any interventions which may be introduced, 3) evaluate the impact of any translocation which may be initiated, and 4) evaluate the potential impact of any unforseen future events such as chemical exposures, biotoxins and environmental changes on health status.

In addition to repeated sampling of seals in each subpopulation once every 2 years, prospective studies include evaluation of individual seals which have been included in translocation and rehabilitation studies and seals opportunistically sampled. Opportunistic sampling will be continued when suitable animals are captured and restrained in the wild for other purposes. This will permit continuous health assessment and prospective monitoring of individual seals. Appropriate identification of individual seals through tagging and radio satellite provides an opportunity to enhance understanding of the long-term effects of disease conditions and the relationship between findings on clinical and laboratory tests and survival. Prospective evaluation of individual seals provides a means to further validate and evaluate the significance of health data that are collected cross-sectionally (at a single point in time).

Seals included for prospective studies include animals captured for epidemiologic, translocation, and rehabilitation studies and seals opportunistically sampled during other management activities (i.e., radio satellite, crittercam deployment and retrieval) and will be followed prospectively to the extent possible. Sampling of these animals will increase our data base and continuous health assessment and monitoring of individual seals (surveillance). These activities provide an opportunity to sample seals more than once (i.e., satellite radio deployment and removal) and follow their status over time. In order to evaluate health and disease status of Hawaiian monk seals longitudinally, improved methods of sampling from living seals in the wild are also needed. A total of 107 seals have been sampled for epidemiologic evaluation following strict protocols and standard techniques. This pilot study provides essential baseline information

regarding the health and disease status of wild Hawaiian monk seals (Aguirre, 1998).

OBJECTIVE 5: DEVELOP PREVENTION AND CONTROL STRATEGIES TO MITIGATE THE EFFECTS OF SUBOPTIMAL HEALTH OF THE HAWAIIAN MONK SEAL

In the context of this Plan, prevention and control are strategies important in enhancing recovery. There has been very little work directed at prevention and control of disease in the monk seal to date. Potential disease management tools include:

Immunization Against Infectious Agents

The immunization of a wild population is not a trivial undertaking and has only been accomplished a limited number of times in any wildlife species, and then, only after extensive evaluation and pilot work. There are issues which merit consideration prior to mounting a program, and are absolutely essential prior to considering going into the field with a "vaccine." Prior to that possibility, questions regarding extent and distribution of antibody and potential for active infections in Hawaiian monk seal populations need to be addressed with better data than currently exist.

However, if one were to consider the immunization of a wild population, a series of steps would be required similar to those used for any biological intended for administration in animal or human populations. Limited published work in this area is based on two animals (Osterhaus, 1997) and does not constitute an adequate data base for field use in Hawaiian monk seals. At a minimum, these steps would include:

(1) Selection of appropriate products(s) accompanied by in vitro testing in cell systems. Assurance of lack of infectivity of killed biologicals.

(2) Establishing antigenicity and immunogenicity of products in non-target species; e.g., laboratory animals or other, non-threatened pinniped.

(3) Establishing safety in non-target species, pinniped.

(4) Pilot testing in target species Hawaiian monk seals (HMS) held under observation for repeated sampling, evaluation of antibody response and other markers; safety evaluation; e.g., Kewalo/San Antonio HMS. Product is assumed to be killed and to have passed safety testing as described above.

(5) Efficacy evaluation of the product in challenge experiments to known or suspected pathogens such as PDV (Phocine distemper virus). Alternatively, if a product has passed safety testing, but in the absence of proven efficacy, one could reason that a

killed product could be used under the "do no harm" notion. However, stress of capture, handling, and release may not constitute a no-risk intervention and a risk/benefit analysis should be conducted.

(6) If prior work shows the approach to be safe, efficacious and feasible, field evaluation of a product could be considered.

Prevention of Infection of Monk Seals with Domestic Animal Agents

The potential for transmission of infectious agents from domestic pets and livestock represents a concern when monk seals are brought into environments where these animals may be found or when personnel bring such animals into the native environment. Several of the agents which potentially have the capacity to infect pinnipeds are found in domestic animals (Canine Adenovirus 1 and 2, Canine coronavirus, Canine parvovirus, and Feline rhinotracheitis virus) but have not been described to date in pinniped populations. Transmission of these agents from domestic animals to Hawaiian monk seals could occur under suitable conditions. As an example, a virus closely related to, if not identical with canine distemper virus, was responsible for the epizootic in Lake Baikal seals that occurred in 1987 and was putatively associated with contact with domestic dogs (Grachev, 1989). Therefore, it is strongly recommended that potential contact between Hawaiian monk seals and domestic animals, especially dogs and cats, be prevented.

Contact is defined as that which might occur directly from animal to animal as well as indirectly through fomites or environmental contamination with infectious material from pets. Animals brought in for testing or evaluation prior to translocation will not be permitted contact with domestic pets. For example, canine parvovirus is an extremely hardy virus which persists in the environment for long periods. The virus is extremely pathogenic in young dogs, causing hemorrhagic gastroenteritis and death. Another parvovirus exists in the cat (feline panleukopenia virus) and is responsible for epidemic mortality in kittens. Introduction of these agents into a naive Hawaiian monk seal population could have disastrous effects and must be avoided. Stringent precautions to prevent transmission of infectious agents from pet animals to Hawaiian monk seals are essential for the recovery of this highly endangered species.

Parasite Control: Prevention/Treatment

The parasites which have been found to infest the Hawaiian monk seal are described above under objective 1. Disease control activities in other mammalian species routinely include parasite control and treatment to reduce burdens; for example, range cattle are routinely treated for parasites prior to moving them into feedlots in areas where parasites are prevalent. Dierauf (1990) lists a number of antihelminthics that have been used in marine mammals with dosages and contraindications. Several of the drugs available have a wide margin of safety and have been recommended by wildlife parasitologists (C. Hibler, pers. commun.) and clinicians for the treatment of monk seals. In addition to their broad spectrum of antihelminthic activity these drugs have a wide margin of safety. Typically, antihelminthics are not 100% effective and do not

remove every parasite; burdens may be reduced by 90%-95% depending on the parasite and drug chosen. Further research in this area and consultation with parasitologists and marine mammal veterinarians responsible for captive pinnipeds are recommended to develop a protocol (drugs, dosage schedules, etc.) for treatment of Hawaiian monk seals to reduce parasite burdens. Monk seals undergoing translocation and rehabilitation are likely candidates for this protocol.

Habitat Manipulation

Habitat manipulation includes removal of sources of anthropogenic pollution and removal of marine debris and foreign materials (i.e., netting, fishline, plastics) in order to reduce the risk of injury, entanglement, or bowel obstruction. Sites that have had extensive human impact are likely candidates for this type of intervention.

In 1982, PSI began documenting Hawaiian monk seals entangled in marine debris. From 1982-96, 139 seals were observed entangled, representing approximately 0.63% of the population (Henderson, 1990). This is the highest rate observed for any pinniped species, significantly higher than the 0.4% for the northern fur seal, a species for which the threat of entanglement has been well publicized. Overall mortality due to entanglement is notoriously difficult to quantify as seals may become entangled and die at sea or during periods when they would not be observed on land. Five Hawaiian monk seal deaths from entanglement were observed, but most of the 139 documented incidents of entanglement resulted in the seal being rescued/released by onsite biologists; lacking human presence, more mortalities would have resulted. Pups and immature seals are particularly susceptible and have higher entanglement rates than adults.

In 1987, PSI began documentation and removal of potentially entangling debris from beaches within the Hawaiian monk seal's geographic range. Through 1996, over 16,700 items were destroyed, most of which comprised small pieces of fishing net or lines of maritime origin. These efforts have been and will continue to be part of the duties of shore-based biologists who monitor monk seal populations. However, logistical and time constraints prevent these staff members from monitoring or cleaning offshore reefs. Preliminary studies have been designed to assess the amount of marine debris attached to coral reefs surrounding reproductive sites and have indicated that entanglement at sea could have a significant impact on population trends of the Hawaiian monk seal.

OBJECTIVE 6: DEVELOP APPROPRIATE CONTINGENCY PLANS IN THE CASE OF UNFORESEEN EVENTS WHICH THREATEN THE HAWAIIAN MONK SEAL

Appropriate contingency and response plans to minimize potential future impacts on the health of monk seals from exposure to anthropogenic contaminants, spills, biotoxins, or natural disasters require development. The development of contingency plans specific for the monk seal is consistent with the broad mission of OPR, NMFS as described in “National Contingency Plan for Response to Unusual Marine Mortality Events” (Wilkinson, 1998). Preparation of these plans

and the setting of response teams to deal with emergencies are especially critical for the Hawaiian monk seal given the highly endangered status of the species. Work under this objective will include:

Implementation of the Hawaiian Monk Seal Unusual Mortality Event (UME) Response Plan

During the December 1997 HMSRT meeting it was recommended that the *Hawaiian Monk Seal Die-off Response Plan, A Workshop Report* (Gilmartin, 1987) be revised and implemented. The Hawaiian monk seal UME Plan will be focused on investigations regarding etiology of a mortality event in the monk seal population, identifying techniques to mitigate or minimize mortality, to provide medical care and rehabilitation to affected live individuals, and to identify the impact of mortality and morbidity on the affected population. These efforts will include a compilation of biological data, problem identification, field investigations, laboratory diagnosis, establishment of control areas, and a communications protocol to address the media. Disease investigations in the monk seal population will focus on determining the causative agent, the pathogenesis of disease, diagnostic methods, the epidemiology of the condition, risk factors leading to the event, and the possible effective treatments or vaccines. In addition, management of unusual mortality events will include methods of carcass removal and disposal, seal translocations, environmental decontamination, surveillance by continuous monitoring, and follow-up investigations. A detailed analysis of the situation and report of the event will provide new insights into future events. Advanced planning will be necessary in any of these situations. Open communication and contact will be established with the Director of the Southwest Region, NMFS. An emergency kit for necropsy, specimen collection, and identification will be available on site at all times and an additional kit may be provided by the Southwest Region. Risk assessment is an important part of the UME Plan. Risk assessment will characterize high risk diseases, infectivity/transmission of agents, prevalence and incidence, fatality and morbidity, availability of preventive measures, diagnostic tests, and public health concerns.

A local group of marine mammal biologists and veterinarians with expertise on biology, diseases, and outbreak responses has been appointed by the Director of the Honolulu Laboratory. The Chief of PSI will be the leader of that team. The UME Team will convene once a year to revise and update the Hawaiian monk seal UME Plan based on the *National Contingency Plan* (Wilkinson, 1996). The team will identify contacts and resources for rapid action in case of a catastrophic event in the population. Pre-event planing, initial steps, administrative tasks and tissue collection, preservation, and shipping to selected laboratories will be evaluated by the team. Any unusual mortality event will be addressed by following well-established protocols supported by this Plan outlining response activities, trained personnel, necessary equipment and supplies, holding/quarantine facilities, and necessary permits.

The UME response team will be composed of at least the following individuals:

(1) The Chief of PSI as leader of the team

(2) A biologist, knowledgeable of Hawaiian monk seal biology, behavior, age

classification, and beach-use patterns in the Northwestern Hawaiian Islands

(3) A member of the Hawaiian Monk Seal Recovery Team

(4) A veterinary epidemiologist knowledgeable in die-off responses, specimen collection and processing, and laboratory techniques in field situations

(5) A veterinary pathologist with experience in post-mortem examination and histopathology with laboratory diagnostic facilities

(6) A representative from the NMFS SWR

Development of the Hawaiian Monk Seal Oil and Chemical Spill Response Plan

Currently the State of Hawaii is developing a response plan to the chemical intoxication of marine wildlife in compliance with the Oil and Pollution Act of 1990. As this plan develops, PSI will collaborate, coordinate, and cooperate with other agencies in the development of a comprehensive and integrative oil and chemical spill response plan for Hawaiian monk seals. Other involved agencies include the Department of Land and Natural Resources of the State of Hawaii, the U.S. Fish & Wildlife Service (in charge of management of the NWHI Refuge System), and the U.S. Coast Guard.

OBJECTIVE 7: INTEGRATE HEALTH AND DISEASE ACTIVITIES WITH THE TRANSLOCATION PLAN OF THE HAWAIIAN MONK SEAL

Work under this objective is directed at integrating the procedures described under objectives 1 and 2 into the translocation efforts described in the Recovery Plan. The purpose of this effort is to enhance success of translocations and to minimize potential disease risks. The general objective is to develop a biomedical plan to be integrated into translocation efforts as a tool for enhanced recovery and population growth. The specific aims under this objective are:

(1) To develop health screening criteria for translocation and to prioritize the components of the baseline screening that will occur prior to translocation.

(2) To recommend additional procedures that will be considered prior to translocation if such gaps are identified.

(3) To present and consider alternative methods for incorporation of screening and medical surveillance within the framework of the translocation process; i.e., screening and holding prior to translocation versus screening and holding at the site of release and individual versus population assessment.

(4) To employ statistical procedures to estimate sample sizes required to minimize risk

of introduction of infectious agents in recipient populations following translocation.

(5) To develop recommendations for future translocations to enhance recovery of the species including donor and recipient populations.

Background and Current Status of Translocation Activities

Translocation is a useful management tool for the recovery of the species. Its potential value for the recovery of the Hawaiian monk seal has been demonstrated both by experience at Kure, and by modeling efforts. Although various methods have been employed, the best method to use is not apparent. Therefore, all translocation, rehabilitation, and conditioning must be viewed as an experiment. Careful experimental design, evaluation of contingencies, minimizing impact on the potential for growth of the source subpopulation, and financial implications must be considered.

Any translocation activity must achieve the goal of supplementing a depleted subpopulation without compromising the potential for growth of the source subpopulation, thereby improving recovery of the total population. Further work is required to predict the best source and destination subpopulations and the best translocation method. At present, however, the management strategy most likely to achieve the goal of subpopulation recovery is the direct translocation of larger sized pups. The strategy recommend by the Captive Care Committee in May 1997 for the immediate future was translocation with or without conditioning on the beach at the capture or release sites. Currently, it is recommended that healthy animals not be transported to Oahu. However, rehabilitation on Oahu remains an option if it is determined to be medically safe and if space and long-term care are available.

Success of a translocation program in increasing the overall total population growth rate depends on an ability to correctly identify source subpopulations where the local prospects for survival are poor and to correctly identify destination locations where the prospects for survival are good. There is limited ability to predict which subpopulations may be the best future sites to release animals or which subpopulations might provide a source of pups for these interventions. This is important because translocated pups may not reach sexual maturity for a number of years, and the future environmental conditions that may prevail at both source and introduction sites cannot be predicted.

At least over the short term, while other focused research is being undertaken, translocating, conditioning, and releasing undersized pups from areas where their probability of survival is low to islands where it may be higher appears to be the most useful intervention that can be undertaken. At present, the most promising source and introduction sites appear to be FFS (unless the eye disease is endemic to FFS and affected pups become blind after translocation) and Midway Island, but this could quickly change as a result of environmental conditions, ecotourism activities, or proposed military operations that cannot be predicted. Similarly, removal of adult males from subpopulations because of skewed sex ratios or aggressive behavior may be

undertaken in some circumstances, and seals must be disentangled from marine debris whenever possible.

The strategy supported by the Captive Care Committee (1997) for the immediate future is direct translocation between two island subpopulations with or without conditioning on the beach of capture or release. This strategy is based on the following premises:

(1) Direct translocation between two sites minimizes stress by reducing time in captivity, handling, and frequency and duration of transport.

(2) There is extensive variability in the survival of pups in any given time period at any given location. For example, recently even heavy pups at FFS have had poor survival when compared to previous time periods.

(3) The biology of the species makes testing of a hard release strategy for newly weaned pups a reasonable research option. However, a soft release may be more effective under some circumstances.

The current guidelines for translocation of Hawaiian monk seals is summarized below. Criteria for selection, holding, and release have been established for other species and were reviewed by The Captive Care Committee (1997).

Selection Criteria

(1) Continue translocation of females.

(2) Seals will be selected on morphological criteria (e.g., size, weight, condition) to maximize the growth of the source and the destination subpopulations. For example, selection of slightly heavier pups will have only a marginal impact on long-term subpopulation trends at FFS.

(3) Choice of source and destination subpopulations will balance the overall goal and logistical realities.

(4) Currently, newly weaned female pups appear to be the best candidates, but other age groups may offer an opportunity for improved experimental design.

(5) Seals will be in good physical health as determined by a marine mammal veterinarian. A physical exam, hematology (CBC, total protein, sedimentation rate), and collection of serum samples for serologic analysis will be performed prior to translocation.

Holding Criteria

While it might be appropriate to release some pups without holding them either at site of capture or site of release (i.e., hard release), others may require some period of holding. If seals are held on site, then the following criteria will be applied:

(1) Feeding protocols will be assessed to determine their relative efficacy. This assessment can be done retrospectively from data already available and experimentally for future releases.

(2) A qualified individual will monitor the health of the animals and care for them. Individuals with appropriate experience might include veterinarians, vet students conducting research, or registered veterinary technicians. Experienced animal caretakers, while not technically trained as veterinary experts, will also be invaluable in assuring the well-being of captive animals.

Release Criteria

Animals will be apparently healthy at release as determined by a marine mammal veterinarian.

Objectives of Medical Screening for Translocation

There are two fundamental reasons for performing medical screening of candidate animals for translocation: to assure that the current health status of the candidate maximizes the likelihood for success in translocation; i.e., that translocation success will not be compromised by pre-existing conditions which impair the ability of animal to withstand the stresses of transport and adaptation to a new environment and to assure that translocation of the candidate will not result in the introduction of new infectious agents into a naive population. The latter consideration is the most critical, since introduction of new agents into recipient populations could have catastrophic implications. While the former is an important consideration with respect to the success of the translocation effort and to avoid the loss of any Hawaiian monk seal, the implications of translocating animals with preexisting conditions that might affect survival is relatively less critical than preventing an epidemic of a new disease in a naive wild population.

Rationale and Justification

The requirement for medical screening of Hawaiian monk seals as a fundamental component of translocation will require little justification. However, to expand on this idea further, the issue of requiring baseline health screening is addressed briefly in this section.

Medical screening contributes substantial costs to translocation, introduces logistical problems, and requires that animals be handled in order to perform procedures and obtain samples. Thus, it could be argued that translocation will proceed without consideration of medical issues; animals in apparent good health on visual inspection would be candidates for translocation. With respect to the possibility of moving disease agents into other subpopulations, one might assume that the entire Hawaiian monk seal population be considered as a unit and that disease agents prevalent in any subpopulation of Hawaiian monk seal would likely exist in all other subpopulations. Theoretically, this premise could be based on the recorded movements of animals from various islands in the Hawaiian Archipelago to others in the chain. This assumption will be rejected on multiple grounds: 1) the distances between subpopulations are great (approximately 800 miles from FFS to Kure Atoll). Records of migration patterns are based on limited number of identified animals that do not assure substantial mixing of animals and introduction of agents has occurred; 2) epizootics of new agents, especially morbilliviruses, are being recognized nearly yearly in marine mammals, indicating the need for real-time contemporaneous monitoring of translocation candidates; 3) new strains of morbilliviruses are being identified rapidly, suggesting the possibility that genetic reassortment or antigenic shift is occurring; 4) the host range of this group of viruses appears to be expanding as evidenced by the agents infecting new species of marine mammals; 5) the development of an ocular syndrome of probable infectious etiology in a group of 12 monk seals translocated from FFS to Oahu provides compelling rationale for the necessity of medical screening; and finally, 6) monk seals are not highly gregarious (like other pinnipeds) which may reduce the potential for horizontal transmission during an epizootic.

Health Screening Criteria and Prioritization of Baseline Data

In order to accomplish this aim, a three-tiered approach was used for the purposes of prioritizing the elements of a baseline screening program for translocation of Hawaiian monk seals. In the scheme described in the following section, only tests and procedures falling into tier 1 would be required prior to translocation.

Tier 1 Tests and Procedures

This group of tests and procedures is considered essential and is required to be completed prior to translocation. Alternatively, and less desirably, screening could be accomplished in a quarantine setting during a holding period on the recipient island associated with a soft release (discussed under Aim 3). The following tests and procedures are considered essential and will be performed prior to eventual release during translocation.

(1) Clinical Exam and Morphometric Measurements.

(2) Hematology and Biochemistry.

(3) Serologic Screening for Antibodies to Infectious Agents: Screening for these agents is required because of 1) their established prevalence in Hawaiian monk

seals or other species of pinnipeds, 2) their established capacity to cause morbidity and or mortality in pinnipeds, or 3) their capacity for horizontal spread within a population. Introduction of any of these agents to a naive population could have negative impacts on the health of translocated animals and could spread horizontally within the recipient population with substantial morbidity and mortality.

(4) Morbilliviruses: Sera from monk seals considered for translocation will be screened for antibodies to the two major viruses that have been shown to infect pinnipeds (PDV and CDV) and cetaceans (PMV and DMV). The presence of antibody to morbillivirus would represent a clear contraindication to translocation and could have serious implications for the Hawaiian monk seal subpopulation from which the samples were collected. The test recommended is the serum neutralization assay as described by Duignan et al. (1997).

(5) Phocine Herpesviruses: Screening for antibodies to phocine herpesvirus 1 and 2 is recommended prior to translocation. Seropositive animals will not be candidates for translocation. Typically, herpesviruses are species (at least order) specific; therefore, the recommendation is confined to testing against the two known strains of phocine herpesviruses.

(6) Brucellosis: Screening for antibody to *Brucellae* is recommended prior to translocation. Hawaiian monk seals with antibody to *Brucellae* will not be considered candidates for translocation.

(7) Salmonellosis: Fecal cultures for *Salmonellae* are recommended prior to translocation. Although the significance of *Salmonella* isolates is not clear, samples and cultures will help reduce the possibility of introducing new pathogenic strains. Further work defining the salmonellae infection status of each of the outlying islands is in progress and will be critical to establishing the endemicity of the agent. Samples have been collected from Pearl and Hermes Reef, Midway, and FFS.

(8) Caliciviruses: Since the pathogenicity of the caliciviruses appears to be low for pinnipeds, the presence of antibodies in the absence of clinical signs is not considered a contraindication to translocation. There is work ongoing to establish the prevalence of antibody to calicivirus for various subpopulations of monk seals (Aguirre, 1998). The currently available data suggest that calicivirus infection is endemic in at least some subpopulations of monk seals. If infection with the agent is documented for all subpopulations of monk seals, then there is little concern that translocation will result in infection of a previously naive population. This recommendation will be finalized when the results of the 1998 sampling and seroepidemiology are known.

A potential problem arises in screening of young seals who may have passively acquired antibody from their mothers. Additional research is required to determine the longevity of passively acquired antibody for the Hawaiian monk seal or other pinniped. This information will be important in determining whether an antibody identified in juvenile, just weaned female pups intended for translocation is of maternal origin, or caused by primary infection of the pup. Specific IgM antibody tests may be available for some agents but are generally not utilized in sero-epidemiologic surveys.

If antibody to an exogenous infectious agent is identified in a translocation candidate, the distinction between passively acquired antibody and active infection may be difficult to impossible to determine. Therefore, the cautious approach is to reject any seal with a positive antibody test (Tier 1).

Maternally acquired antibody titers will wane and disappear over time, while those due to an active infection will persist. Unfortunately, this strategy does not lend itself well to the translocation scenario. Therefore, it is recommended that juvenile animals with antibody to Tier 1 agents not be translocated without further evidence to show that they are uninfected. Differentiation between IgM and IgG antibody is a potential means of partially differentiating maternally derived antibody from that obtained by active infection. (IgG may be of maternal origin or the result of an active infection occurring within approximately 14 days). The availability of specific IgM tests would have to be determined on an agent-by-agent and laboratory-by-laboratory basis; this technique is not likely to be useful across a broad array of agents for which screening is recommended.

Tier 2 Tests and Procedures

This group of tests and procedures would provide useful information but is of less critical importance; animals could be translocated without results yet available or without performing the test. At present, there is inadequate evidence that these agents are prevalent in pinniped populations or that they pose a substantial risk for translocated Hawaiian monk seals under the conditions described in the report. The following tests and procedures fall under tier 2 in the prioritization of procedures required or recommended for screening candidates for translocation.

(1) Influenza virus

(2) Sea lion adenovirus

(3) *Chlamydia psittaci*

(4) *Toxoplasma gondii*

Tier 3 Tests and Procedures

This group of tests and procedures has potential importance as a research tool and will be performed where feasible; however, these tests are not required for translocation.

(1) Hormonal evaluations for reproductive status and growth: These hormones may be useful tools for predicting patterns such as male aggression (testosterone) or growth. However, at this point they are considered research objectives and are not required for translocation.

(2) Characterization of body burdens of contaminants: Especially organochlorine (OC) pesticides and polychlorinated biphenyls (PCBs). These lipophilic compounds can be assayed in blubber samples or whole blood. There is substantial interest in these compounds due to their well-known effects on reproductive success and immune function in other species. Recent research has established the capacity of some organochlorines to modulate estrogen or androgen hormones and metabolism. The contaminant exposure of the Hawaiian monk seal, therefore, becomes an important component of predicting reproductive success for the species as well as for the potential impact on immune responses to infectious agents. Also, it has been recognized that DDEs transfer from mothers to pups in other species of pinnipeds, especially after the first pregnancy. Further research on the distribution and effects of OC and PCB contaminants for the Hawaiian monk seal is strongly recommended, but is not considered a requirement for translocation.

Additional Procedures

A major issue that requires further investigation is the cause of the ocular condition which developed in the captive juvenile monk seals at KRF from 1995-98 (Aguirre et al., 1998). This has major significance since 1) the seals with the eye condition originated from FFS; 2) FFS will be the first likely source of translocation candidates in the future; 3) conjunctivitis was the first clinical sign of the syndrome, and was reported for 2 seals on FFS prior to transport to Oahu for rehabilitation (other seals developed conjunctivitis during the first few weeks after their arrival to Oahu); therefore the agent responsible for the ocular syndrome was possibly present in some of the seals on FFS; and 4) if, as suggested above, this condition is due to reactivation of a latent herpesvirus or other agent, then future movements of monk seals from FFS may result in the same outcome and be contraindicated. Therefore, it is recommended that continued efforts be made to identify the agent(s) responsible for the ocular condition.

Individual Versus Population Assessment

In order to accomplish the goals of medical screening described under Aim 1, two alternative strategies exist: population and individual assessment.

Population Assessment

Population assessment relies on prescreening at the population level to assess the prevalence of infectious agents. For example, if an appropriate number of monk seals were

screened for antibodies to morbillivirus on FFS and none were found, the assumption could be made that seals on FFS are not infected with this agent within the limits of statistical certainty described in the next section. Therefore, prescreening of a donor population could be accomplished to assure that the agents described under Tier 1 are not circulating in this population. The advantage of this approach rests in the ability to move animals rapidly from the donor location to the recipient site, without the requirement for an extended holding or quarantine period at either the donor or the recipient site. This approach would facilitate a hard release or direct translocation strategy without a lengthy holding period.

There are several disadvantages associated with this approach. First, a two-stage process is required in which an adequate number of seals must be captured, bled, and released prior to the translocation effort. The size of the sample required is discussed below under aim 5. Second, there is at least a theoretical possibility that the probabilistic sample drawn may fail to identify an infected animal. This could occur because the agent is not uniformly distributed among age classes of animals and the sampling fails to adequately account for such distributions. Third, the longer the time elapsed between the initial screening of the population and the selection of animals for translocation, the higher the probability becomes that the antibody free status of the screened population is no longer valid. This is of particular concern for the morbilliviruses, where cetaceans as well as pinnipeds have been shown to be infected and may play a role in introduction of agents to naive populations. If for example, seals on FFS are screened and found to be free of antibody to morbillivirus, how long does their morbillivirus free status exist? Periodic rescreening would be required to assure that the agent had not been recently introduced. Although there are no firm guidelines for the interval required for rescreening, a conservative approach based on disease control programs for domestic animals might call for annual rescreening. In that case, much of the advantage of relying on population assessment, rather than individual assessment is lost. Fourth, this strategy is best suited to the hard release scenario. If the decision is made to use soft release techniques to afford the translocated animals a period of acclimatization or supplemental feeding, then a predesignated holding period will facilitate a health evaluation or screening for infectious agents. A soft release technique should encompass an adequate holding period to accomplish a sufficient evaluation (14 to 21 days) while meeting the other objectives of the soft release strategy.

An additional potential use of population assessment is to determine endemicity of an infectious agent (rather than freedom from prior exposure). This has two potential advantages. First, if an agent is found to be endemic in both the prospective donor and the prospective recipient population, then the risk to the recipient population from a translocation is reduced since the population is not naive. Second, if a donor population is found to be free of an infection which is endemic on a prospective recipient population, then the risk of acquiring the infection after translocation needs to be considered. The specific risks of morbidity and mortality will then be considered for each age and gender group. For this example, translocation of a pregnant female would be contraindicated. Therefore, the population assessments for infectious agents will be continued, but will not replace the need for individual assessment of prospective subjects for translocation.

Individual Assessment

From the foregoing discussion, the advantages to using an individual screening approach to assure protection against introduction of infectious agents into naive populations become clear. First, no prescreening of the donor population is required since each prospective candidate for translocation is evaluated individually prior to release. Second, the interval required for rescreening (annual, biannual) becomes unnecessary. Third, there is no reliance on statistical probability of detecting an infectious agent when the prevalence is low. As shown below, the sample size requirements to assure freedom from infection when the prevalence of an agent is low are considerable, and may exceed the number of animals available for screening. Fourth, the costs associated with individual animal screening would be lower since only the animals intended for translocation need to be screened. As an example, there would be no requirement to screen 40 animals (population assessment) if the goal is to translocate only 10 individual seals. Fifth, the need for excessive handling of animals for population screening is removed, minimizing the risk of injury. Finally, the individual screen-and-release strategy would require a holding time of 14 to 21 days providing a quarantine period for the detection of diseases which are in the incubationary stage or which are due to reactivation of a latent carrier state. For the reasons presented above, it is recommended that screening for infectious agents prior to translocation be conducted on an individual basis rather than relying on prescreening and probabilistic sampling of the donor population.

Alternative Methods and Screening Holding Site

As described above, if individual screening for infectious agents is chosen to assure that an animal is healthy and free from infection with selected agents, then a holding period is required prior to release. During the holding period, the samples would be flown from the donor island back to Oahu and then transhipped to a limited number of collaborating laboratories. A pre-arranged agreement with the laboratories would be in place to assure immediate processing of all samples, with notification of results by telephone to minimize the holding time required. With appropriate logistical support and coordination between the field personnel and the laboratories, a holding time of 14 days with an upper limit of 21 days will be realistic to conduct well-established serological tests, or bacterial cultures. Note that the protocols recommended do not require virus isolation attempts, a much lengthier and complex procedure.

Two alternative strategies exist for the holding period. These follow the strategies previously identified for soft release. Seals could be held in enclosures at the site of capture on the donor island under quarantine, transported to the recipient island and released (hard or soft) or transported to the recipient island after capture and held during the quarantine period for testing results at the site of release.

From a medical and epidemiological perspective, holding and quarantine at the site of capture has several advantages over holding prior to release at the donor site. Principally, it will be difficult to contain an infectious agent if animals are moved to the donor site and are shedding such an agent. Animals held in enclosures at the recipient site may still present an opportunity for

horizontal transfer of agents to the recipient population. This could occur by inadvertent contact with resident animals, by environmental contamination and future contact with infective materials, by fomites such as those used by personnel caring for the animals in the pens, or by indirect transmission through birds or indigenous wildlife. The enclosures designed for holding during a soft release will not meet full, strict quarantine objectives. Strict quarantine would be impossible to accomplish under beach conditions, but limited quarantine could be achieved by suitable design of holding pens. Finally, animals found to be ill, not suited for translocation, or infected with any of the tier 1 agents, could be treated, rehabilitated, and returned to their native environment without risking the health of seals on the recipient island.

Therefore, from a medical perspective, it is recommended that the holding and quarantine period required be the site of capture. This assures that a seal is healthy and not carrying infectious agents which may pose a risk to the recipient population. During this period, medically trained personnel would observe seals daily for signs of clinical illness, perform physical examinations, hematological and biochemical evaluation, evaluate for parasites, perform fecal cultures, and screen for antibodies to selected infectious agents.

Following the holding period, the seals found to be suitable candidates for translocation would be moved to the recipient site. At that time, a further decision for hard or soft release would be made and based on considerations such as the animal's ability to forage independently, its weight and body condition, and requirements for an acclimatization period. The length of an additional holding period could be tailored to the individual animal's requirements and would not be dependent on the receipt of additional medical data or pending tests, thus providing maximal flexibility in the release schedule for each seal.

Statistical Procedures for Sample Size Requirements

As pointed out under aim 3, estimation of required sample size for population screening for prevalence of antibody to selected infectious agents becomes an issue only if the population assessment strategy is adapted for translocation. If individual assessment is conducted prior to translocation, the requirement for prior population screening is removed, decisions are based on data from the individual animal, and the sample size considerations are not relevant. However, the need to screen populations may also be justified when there is need to determine the role of health and disease in population trends.

A summary of required sample sizes to detect a varying prevalence of infection is presented in Table 3. The entries in the table show the probability that a diseased animal will not be found for that prevalence and sample size. For example, if the disease has a prevalence of 5% and 10 animals are screened, there is a 60% probability that no diseased animal is found and a 40% probability that a diseased animal is found; if the prevalence is only 1%, then the chance of finding a positive animal if only 10 animals are screened is only 10% (1.0-0.90). Note that this table assumes that if antibodies are present they are distributed uniformly across the age groups of pup, juvenile, subadult, and adult and the sampling of animals would be proportional across age groups. If this assumption is incorrect; i.e., if antibody prevalence is higher in adults and lower in

pups for example, then the sampling strategy would need to be adjusted to maximize the probability of detecting infected individuals in the population. This would require an increase in sample size to assure adequate representation within age groups.

To further illustrate the sampling strategies required, suppose a reasonable sample size (based on personnel and other resources) that could be collected from a prospective donor population is 40. If 40 seals were collected and their sera examined, one would expect to detect antibody against a specific agent if 20% of the animals were infected (Table 3). If 5% of the animals were infected, the probability of detecting an infected individual is still good (1.0-0.13 = 87%). If, however, the prevalence of infection is 2% or less, then the probability of detecting an infected seal is only about 50:50 or poorer. If the prevalence is only 1%, then there is only a 33% chance that an infected animal will be found if 40 animals are sampled. Sampling approximately 100 animals is required to maintain the probability of detecting a 2% prevalence of infection with over 85% certainty. When attempting to minimize the risk of introducing infectious agents into naive populations, the probability of detection must be greater than 85%. Note that the issue here is not to determine whether the seals being screened are actually shedding the organism. The data provided in Table 3 reflect only the ability to detect prior infection in the prospective donor population and are based on the assumption that one would not translocate seals coming from a population known to have been infected with one of these agents to a population that has not been exposed to one of these agents.

The data presented in Table 3 and the discussion above provide further rationale for the recommendation that individual, rather than population, assessment be used to make decisions about the suitability of prospective donor populations for translocation.

It is well known that diagnostic tests may produce incorrect results. The ability of a diagnostic test to accurately identify a diseased or infected individual is known as its sensitivity. The ability of the test to accurately identify a non-diseased or non-infected individual is known as its specificity. Suppose that a test to be used to screen for a location is not 100% sensitive; i.e., false negatives occur. What are the consequences in terms of sample size that might be required to assure that an island's seal population is free of disease? The following section addresses this issue (T. J. Keefe, unpubl. data, 1998).

Table 3. Sample Size Requirements for Detection of Antibody to Selected Infectious Disease Agents. Probabilities are for Failure to Detect An Infected Animal by Prevalence in the Population and Sample Size.

Sample	Prevalence of infection (%)						
size (*N*)	0.1	0.2	1.0	2.0	5.0	10.0	20.0
10	0.99	0.98	0.904	0.817	0.599	0.349	0.107
20	0.98	0.961	0.818	0.668	0.358	0.122	0.012
30	0.97	0.942	0.74	0.545	0.215	0.042	0.001
40	0.961	0.923	0.669	0.446	0.129	0.015	0
50	0.951	0.905	0.605	0.364	0.077	0.005	0
75	0.928	0.861	0.471	0.22	0.021	0	0
100	0.905	0.819	0.366	0.133	0.006	0	0
125	0.882	0.779	0.285	0.08	0.002	0	0
150	0.861	0.741	0.221	0.048	0	0	0
175	0.839	0.704	0.172	0.029	0	0	0
200	0.819	0.67	0.134	0.018	0	0	0.00

Sample size determination for detecting disease prevalence

Let *N* denote the population size and
P denote the population prevalence of a disease.

For a sample of size *n* from the populations let *X* denote the number of animals that test positive. For any one animal, the probability of testing positive is given as:

P^+ = P(pos-test/disease) x P (disease) (1)
+ P(pos-test/no disease) x P(no disease)
= Se x P + (1-Sp) x (1-P),

where Se and Sp denote the sensitivity and specificity of the test, respectively.

If the test is 100% sensitive and specific, then (2)

$P^+ = P$; and even if $P = 0$, $P^+ = (1-Sp)$.

Thus, if the test results for the *n* animals can be assumed to be independent, then *X* has a binomial

distribution with parameters *n* and P^+.

For large *n*, *X* is approximately distributed as a Poisson variable with intensity parameter (mean) *u*, so that

$$P(X\ -k) \cong \frac{e^{-\mu}\ \mu^k}{k!,} \quad (K = 0, 1\text{---}) \qquad (3)$$

where $\mu = nP^+$. (4)

So, $P(X>0) = 1-P(X=0)$

$= 1-e^{-nP+}$.

Thus, the level of significance of the test of Ho: P = 0 (which is rejected if X>0) is equal to $\propto = P(x>0/P=0) = 1-e^{-n(1-Sp)}$ and the power of the test is equal to: (5)

$$Pwr = P(X>0/P>0) = 1-e^{-n[SeP\ +(1-Sp)\ x(1-P)]}. \qquad (6)$$

Hence, any $\propto$-level test with poser greater than or equal to $\propto$ must satisfy:

$$n(1-Sp) \leq -\ln (1-\propto), \qquad (7)$$

and

$$n[Se^xP + (1-Sp)x(1-p)] \geq -\ln (1-\propto). \qquad (8)$$

Assume that the test is 100% specific (i.e., Sp = 1).

Then, inequality (4) is satisfied for any value of *n*, and inequality (5) reduces to

$$n\ Se^xP \geq -\ln(1-\propto), \qquad (9)$$

or equivalently

$$n \geq \frac{-\ln(1-\propto)}{Se^xP} \qquad (10)$$

$$= \frac{-\ln(1-\propto)}{Se}. \qquad (11)$$

Consequently, sample size calculations based on an assumed sensitivity of 100% must be increased by a factor equal to (1/Se). [See Table 4.]

Table 4. Sample Size Requirements for Prevalence at Varying Levels of Sensitivity of a Diagnostic Test for Detecting Antibody to an Infectious Agent.

		Prevalence		
Power	**Sensitivity**	0.10	0.01	0.001
0.80	1.00	16	161	1609
	0.95	17	170	1694
	0.90	18	179	1789
0.90	1.00	23	230	2303
	0.95	24	244	2424
	0.90	26	256	2558

OBJECTIVE 8: INTEGRATE HEALTH AND DISEASE ACTIVITIES WITH THE REHABILITATION PLAN OF THE HAWAIIAN MONK SEAL

Develop Recommendations for Future Rehabilitation Efforts including Possible Rehabilitation Sites and Evaluation of Risk

There is a growing body of literature relevant to the issue of rehabilitation from an epidemiologic and medical perspective. As newly identified infectious disease agents have emerged, especially multiple morbillivirus strains, extensive consideration has been given to the risks involved in releasing stranded marine mammals back into the natural environment after spending time in captivity. Many of these issues are summarized in recent reports: *Draft Release of Stranded Marine Mammals to the Wild: Background, Preparation, and Release Criteria* (NMFS, FWS, 1998), *Rescue, Rehabilitation and Release of Marine Mammals: An Analysis of Current Views and Practices* (St. Aubin et al., 1996). These reports contain sections on release guidelines for stranded pinnipeds, cetaceans, sea otters, and sirenians and form a framework for evaluating the recommendations in this Epidemiology Plan. In addition, specific release guidelines for pinnipeds have been obtained from the Marine Mammal Center (Frances Gulland, pers. commun., 1998) to provide additional perspective in addressing the critical components of a translocation program with respect to medical screening during rehabilitation.

Since 1981, captive care and release programs have been an integral part of management efforts to conserve the Hawaiian monk seal. Three strategies have been used, including on-site protection and release, direct translocation from one site to another, and transport to Oahu for rehabilitation, followed by release into a depleted wild subpopulation. The initial intent of captive care and release programs was to enhance the depleted subpopulation at Kure Atoll. So far, the donor subpopulation for all translocated pups has been FFS. In the mid 1980s, half of the entire species was found at FFS, and half or more of the total pup production occurred at that site. In the

late 1980s and early 1990s, juvenile survival at FFS fell sharply, and even the largest pups had only a 30% to 40% chance of surviving to age-2. Further, age at first reproduction for females at FFS has increased to age 8 or 10. Therefore, the intent of the captive care and release program was extended to include salvaging of the reproductive potential being lost at FFS. In the early 1990s, however, captive care and release efforts were small relative to the numbers of seals being lost at FFS. In 1995, PSI and Sea Life Park Hawaii attempted to expand the rehabilitation capacity and thereby increase the ability to mitigate the losses occurring at FFS. In 1995, twelve pups were captured at FFS for rehabilitation on Oahu with the goal of returning them to the wild at Midway Atoll. These seals were not released as anticipated due to a persistent eye condition of unknown etiology (Aguirre et al., 1998). This condition has not been previously reported in wild or captive monk seals. Reinstatement of rehabilitation efforts at Oahu will partly depend on the characterization of the etiologic agent(s) responsible for the ocular syndrome, relocation of affected seals to another facility, and proper rehabilitation and quarantine protocols.

EPIDEMIOLOGY PLAN ACTIVITY SCHEDULE AND TASK PRIORITY

The 5-year (1998-2002) schedule of the Hawaiian monk seal epidemiology plan activities is outlined in Table 5. The priorities assigned to these tasks were based in recommendations of the Recovery Plan, the Recovery Team, and the Captive Care Review Panel. The donor population for translocation and rehabilitation sites has been identified at FFS at this time. The subpopulations identified as suitable recipient populations include Midway and Kure. The costs allocated to the die-off response plan and the oil and chemical spill response plan are considered emergency funds that may be carried over for the following year if not used. If funds are allocated, retrospective studies are expected to be completed by year 2000. The increase in JIMAR Grants for fiscal year 1999 and beyond correlates with the hiring of one field person to support Translocation and Rehabilitation. Although funds for translocation and rehabilitation include limited funds for travel by aircraft charter, it is expected that the NOAA ship *Townsend Cromwell* will be available at no cost to the Epidemiology Task to provide logistical support in the field within the time frames required. Additional funds will be needed if aircraft charters are required for unscheduled trips and to complete the epidemiological activities scheduled on this plan. The estimated inflation annual rate was not included in these figures.

MILESTONES, PRODUCTS, AND SCHEDULES

Completion of pilot study for translocation evaluation	Fall 1998
Completion of Epidemiology Plan	Spring 1999
Appointment of unusual mortality events team	Summer 1999
Revise and implement unusual mortality response plan	Fall 1999
Data base and library	Fall 1999
Preliminary translocation evaluation	Summer 1999
Develop oil and chemical spill response plan	Spring 2000

Rehabilitation efforts begin	Summer 2000
Retrospective studies completed	Fall 2000
Progress reports	Winters 1998-2002

Table 5. Outline of the Hawaiian monk seal epidemiology plan and estimated associated costs in thousands, 1998-2002

TASK ACTIVITY	RESEARCH PRIORITY	FISCAL YEAR	FIELD SITE	ESTIMATED FUNDS 1998	1999	2000	2001	2002
Pilot study for translocation	high	1998-1999	FFS, P&H	18.5	25	-	-	-
Translocation studies	high	1999-2002	FFS&?	-	25	50	50	50
Rehabilitation in situ	high	2000-2002	FFS&?	-	25	50	50	50
Dieoff response plan	high	1999	All	-	25	25	25	25
Oil and chemical spill response plan	moderate	2000	All	-	25	25	25	25
Prospective studies	moderate	1998-2002	All	-	5	5	10	10
Retrospective studies	low	1998-2000	All	-	10	10	-	-
Specimen testing	high	1998-2002	All	19.7	30	30	30	30
Blood/serum and tissue specimen banks	high	1998-2002	All	-	5	5	5	5
Research contracts	high	1998-2002	All	35	50	50	50	50
Data base and library	moderate	1998-2002	All	-	1	1	1	1
Grants--JIMAR	high	1998-2002	-	72.6	130	140	150	160
Subtotal				145.8	356	391	396	406
Overhead (11%)				17.5	39	43	44	45
TOTAL				163.3	395	434	440	451

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APPENDIXES

APPENDIX I: DEFINITIONS

Control--Mitigation of effects of diseases which are currently prevalent in the population and contribute to morbidity, mortality, and suboptimal growth and reproduction.

Disease--Any impairment that interferes with or modifies the performance of normal functions, including responses to environmental factors such as nutrition, toxicants, and climate; infectious agents; inherent or congenital defects; or combinations of these factors. Infection with parasites is ubiquitous in wildlife and reference is made to normal parasite burdens; that is, parasites at this level have little or no impact on the host. The actual effect of these parasites on the animals, as is the case of monk seals, is largely unknown.

Disease investigation--Process of understanding of disease to eliminate or control the spread of that disease within and among populations. Diverse components of health and disease investigations can be applied following the epidemiologic methodology. The first stage is the collection of relevant data. Disease investigations can be qualitative or quantitative or a combination of both approaches. In order to assess the effects of disease on the monk seal population, epidemiologic investigations determine origin of unknown etiology, collect information on natural history and ecology, develop and monitor unusual mortality events responses, and assess current and alternative control programs.

Disease monitoring--Systematic collection of biomedical data to identify diseases in a population.

Disease surveillance--Continuous assessment of the status of specific diseases in a population by close observation of individual seals or groups of seals.

Epidemiology (from Greek *epi* = upon; *demo* = people, *logo* = study)--Study of disease in populations and the factors that determine its occurrence.

Epizootiology--Study of disease in animal populations and the factors that determine its occurrence have a similar meaning with reference to animals; however, the more common term will be used in this document. The general pattern of disease within the population can be described as *enzootic*, a disease that occurs in the population at a regular, predictable, or expected rate. An *epizootic* occurs when a disease appears at a time and place where it does not usually occur, or with a greater frequency than normal. The less precise terms *outbreak* or *die-off* refer to many cases occurring within a short time and are not necessarily synonymous with epizootic.

Hard Release--Immediate release of translocated pups to a new location either within or outside of the atoll or island of birth.

Health--State of morphologic, physiologic, and psychologic well-being or homeostasis and productivity including reproduction.

Health indices--Easily observed parameters that can be used as a guide to the state of health of an individual or population.

Mobbing--Aggressive behavior exhibited by multiple males attempting to mount an adult female or juvenile of either sex.

Predictive values--Probabilities which provide answers to questions regarding the proportion of test-positive animals truly infected and the proportion of test-negative animals truly noninfected. These values depend on sensitivity, specificity, and prevalence.

Prevention–Strategies directed at protecting populations of currently unaffected monk seals from the effects of diseases which are not currently endemic.

Sensitivity--Probability that a test will correctly identify animals which have been exposed to a given pathogen (true positives). False negative reactions may occur because of natural decrease of antibody over time, antibody destruction due to improper handling of specimens, establishment of the threshold titer value at a level that is too high, and improper evaluation of the laboratory test.

Soft Release--Delayed release of translocated pups after temporary holding to acclimate at a new site either within or outside of the atoll or island of birth.

Specificity--Probability that a test will correctly identify animals which have not been exposed to a given pathogen (true negatives). False positive reactions may be present due to nonspecific proteins or substances reacting to the test and incorrect evaluation of the test.

Unusual Mortality Events (UME)--Unexpected mortality; any unusual morbidity or mortality cases in a Hawaiian monk seal population that can be explained as *outbreaks*, *die-offs*, or *epizootics* and demand immediate response. This response is justified even if small numbers of seals appear to be affected.

APPENDIX II: INFECTIOUS AGENTS

VIRUSES

Morbilliviruses

Infection with morbilliviruses is considered to hold the most devastating potential impact for the long-term survival of the Hawaiian monk seal. Introduction of a morbillivirus to a naive population of Hawaiian monk seal could be catastrophic for the recipient population, and therefore for the species as a whole. Morbillivirus infection is also a major threat for the Mediterranean monk seal (*Monachus monachus*) (Osterhaus, 1992). The first known morbillivirus epizootic of marine mammals was associated with a tenfold increase in strandings of bottlenose dolphins (*Tursiops truncatus*) during the 1987-88 die-off along the Atlantic coast (Geraci, 1989; Lipscomb et al., 1994). A second mass stranding of bottlenose dolphins was subsequently recorded and documented during 1993-94 in the Gulf of Mexico (Lipscomb, 1994; 1996). In 1987, a morbillivirus which appeared to be canine distemper virus (CDV) killed thousands of Baikal Seals (*Phoca sibirica*) in Lake Baikal, Russia (Grachev et al., 1988). In 1988, a mass die-off of over 17,000 harbor seals (*Phoca vitulina*) occurred off the coast of northwestern Europe, attributed to a morbillivirus later designated phocine distemper virus (PDV) (Kennedy, 1988; 1989). A morbillivirus closely related to pest des petites ruminants, a virus of domestic herbivores, killed thousands of striped dolphins (*Stenella coeruleoalba*) in the Mediterranean Sea in 1990 and 1991, and was later designated dolphin morbillivirus (Domingo, 1990). A fourth morbillivirus was isolated from porpoises and designated porpoise morbillivirus (PMV) (Kennedy et al.,1992). The two cetacean strains appear to be more closely related to each other than PDV and CDV, which have primarily infected pinnipeds (Barrett et al., 1993). As shown by serologic studies in manatees (Duignan et al., 1995) and Atlantic walruses (Duignan et al., 1994), these agents have a wide host range across the orders of marine mammals. Antibody titers to porpoise morbillivirus and dolphin morbillivirus have been found in sera of pilot whales (Duignan et al., 1995). Antibodies to PDV have been found in harp seals (Phoca groenlandica) and ringed seals (*Phoca hispida*) in Greenland collected prior to the 1988 harbor seal epizootic (Duignan, 1997) as well as in harbor seals and gray seals (*Haliichoerus grypus*) along the Atlantic coast (Duignan et al., 1997), illustrating the wide geographic distribution and host range of this group of morbilliviruses. Antibodies to PDV were not detected in serum collected from 80 Pacific harbor seals along the northwest coast during 1992-93 (Duignan et al., 1995). However, serological evidence of morbillivirus infection was recently found in small cetaceans from the Southeast Pacific, thus providing a potential pathway for transmission to the Hawaiian monk seal (Van Bressem, 1998).

Phocine Herpesvirus

Phocine herpesvirus was first identified as a cause of morbidity and mortality during an epizootic in harbor seals in 1985 (Osterhaus et al., 1985); 11 of 23 affected seal pups died. The organism from that epizootic has since been identified as phocine herpesvirus-1. A second seal

herpesvirus (phocine herpesvirus-2) has been isolated from a captive California sea lion (Kennedy-Stoskopf et al., 1986), free-ranging harbor seals from the North Sea (Lebich et al., 1994) and free ranging harbor seals from the North Atlantic offshore the United States (Harder et al., 1996). Collectively, the herpesviruses are usually associated with lesions in the oral and nasal mucosae, pneumonia, ocular disease, and abortion. Neurotrophic strains of herpesvirus are common and have been described in a harbor porpoise (*Phocaena phocaena*) (Kennedy et al., 1992).

Antibody to the phocine herpesviruses appears to be widely distributed; antibodies to PHV-1 have been detected in the Antarctic from Weddell seals (*Leptontchotes weddelli*) and in the Arctic from harp and hooded seals (*Cystophora cristata*) (Gulland, 1997). In a recent study conducted in Alaska, antibody to PHV-1 was found in 29%-77% and to PHV-2 in 16% to 50% of a sample of pinnipeds collected from 1978 to 1994 (Zarnke, 1997). Species represented included walrus (*Odobenus rosmarus*), northern fur seal, harbor seal, spotted seal (*Phoca largha*), ribbon seal (*Histriophoca fasciata*), Steller sea lion (*Eumetopias jubatus*), bearded seal (*Erignathus barbatus*), and ringed seal (*Pusa hispida*). During a stranding event of 700 live Pacific harbor seal pups on the central and northern coast of California, 379 pups died. Approximately half of these seals that were examined had lesions suggestive of a herpesvirus including adrenocortical and hepatic necrosis associated with intranuclear inclusions and electron microscopic evidence of herpesvirus-like particles. A herpesvirus-like virus was isolated from adrenal tissue on cell culture (Gulland, 1997).

Influenza Virus

Influenza A viruses were first isolated during an epizootic of pneumonia in harbor seals in 1980 off the New England coast and again in June 1982 and March 1983 (Geraci, 1982). A third influenza virus was isolated from a pilot whale (G*lobicephala melaena*) which stranded off the coast of Maine in 1984 (Hinshaw, 1986). The virus isolated from seals and the pilot whale was antigenically and genetically similar to avian influenza virus strains. Since these early descriptions, there have been several minor outbreaks in harbor seals along the Atlantic coast with several different strains of influenza virus (Callan, 1995). Although the extensive avian populations which inhabit many of the islands occupied by monk seals give rise to concern that genetic reassortment of an avian influenza virus might lead to an epizootic in Hawaiian monk seal, there has been no evidence of influenza virus activity in Pacific pinnipeds. Little evidence of influenza virus activity has occurred since the original descriptions in the early 1980s. Influenza viruses typically have extremely short incubation periods; for the harbor seals it was approximately 3 days. Further, influenza viruses are not associated with chronic carriers; they are easily transmitted by direct contact from host to host without becoming established in carrier animals or reservoirs. Thus, seals with influenza virus infection will become clinically ill during a holding period of a week or more.

Calicivirus

Caliciviruses have been identified relatively frequently in pinnipeds by serological screening as well as by isolations from lesions. Twenty-eight serotypes of calicivirus are available for screening. Many of these have been isolated from marine mammals including California sea lions, northern fur seals, northern Elephant seals (*Mirounga angustirostris*), Pacific walrus (*Odobenus rosmarus*), Steller sea lions, and Atlantic bottlenose dolphin (Kennedy Stoskopf, 1990). The marine caliciviruses appear to be serotypes of vesicular exanthema of swine virus and the relationship between the swine disease in California, and the isolation of the San Miguel sea lion virus off the coast of California has been well described (Smith et al., 1973). Calicivirus infection in marine mammals of coastal California appears to be endemic and may be related to infection in the Opaleye perch, which overlaps the host range of the California sea lion (Kennedy Stoskopf, 1990).

Sera from all 10 pups sent to Oahu for rehabilitation were positive for calicivirus using an immunoblot procedure. Similarly, immunoblot preparations for calicivirus group antigens were positive in most of 19 seals captured on FFS during April 1992; however, serum-neutralizing tests for antibody and repeated culture attempts were negative. Calicivirus induces vesicular lesions on the skin of infected animals; in pinnipeds, these typically occur on the dorsal surfaces of the foreflippers (Kennedy Stoskopf, 1990).

Sea Lion Adenovirus

Infection with the sea lion adenovirus has been described in two case reports in California sea lions (Dierauf, 1990). Six animals were affected; the disease induced was uniformly fatal. The virus induces hepatic necrosis and appears to be related to canine adenovirus-1. Thus, the likely source of infection for the California sea lions is contact with dogs. When adenovirus-affected sea lions were brought to marine mammal centers where numerous other pinnipeds were housed, there was no evidence of horizontal transmission. The agent may produce subclinical infections.

BACTERIA

Brucellosis

Brucellosis is an important infectious disease of many mammalian species including humans. Infection with brucellae is typically followed by abortion or stillbirth and by epididymitis and infertility in males. The disease is spread horizontally by contact with infective discharges from aborting females, by ingestion, and by other routes. Brucellae have been recently identified in marine mammals; *Brucella* strains have been isolated from common seals, a grey seal, a hooded seal (*Cstophora cristata*), and several species of cetaceans (Ross, 1996; Foster, 1996). A similar *Brucella* species was isolated from the aborted fetus of a bottlenose dolphin

along the California coast (Ewalt, 1994). The strains do not appear to be members of known species of the organism, and a new species has been proposed (Jahans, 1997). Recently, titers against brucellae were detected in 18 of 102 Pacific harbor seals and 4 of 50 California sea lions from Puget Sound, Washington, indicating relatively widespread infection among Pacific coast pinnipeds (Lambourn, 1996). The organism was also recently isolated from *Parafilaroides* lungworms in a Pacific harbor seal suggesting a potential role for the parasite as a secondary mechanism of transmission (Garner, 1997).

Leptospirosis

Leptospirosis has been reported in three species of pinnipeds from the Pacific coast of North America. The disease was first described in California sea lions (*Zalophus californianus)* along the California and Oregon coasts in 1970 (Vedros et al., 1971 during a major epizootic. Most literature descriptions of leptospirosis in pinnipeds are of *Leptospira pomona*. Antibody to *L. pomona* was associated with abortion, prematurity and stillbirth (Smith et al., 1974) on San Miguel island. In Northern fur seals, the same agent was suggested to be responsible for a syndrome characterized by multiple hemorrhagic lesions in perinatal seals (Smith et al., 1977). More recently, *L. pomona* has been responsible for three sea lion epizootics along the central California coast, characterized by a high prevalence (33%) of renal disease leading to death in over 70% of affected animals. A 1996 survey of 225 California sea lions found 38% of the animals to be positive, with the highest seroprevalence rates among subadult males (Colagoross-Schouten et al., 1998).

The recent account of infection with renal disease due to *L. grippotyphosa* in Pacific harbor seals undergoing rehabilitation (Stamper et al., 1998) adds to the need to broadly screen across the serovars of this organism. Sera from the 12 pups sent to Oahu for rehabilitation in 1995 were negative to leptospiral antigens at a titer of 1:100. In earlier surveys, antibodies to leptospira were detected in Hawaiian monk seals from FFS at low titers (1:100). The significance of these low titers was considered questionable since they occurred in the absence of clinical signs or pathologic evidence of disease and since titers found during epizootics of leptospirosis among California sea lions were much higher (Gilmartin, 1993). It is anticipated that some antibody may be found among potential candidates for translocation and it is recommended that such animals not be moved. Urine culture or identification of leptospira in urine by phase contrast or dark field microscopy is advocated by some as a tool to determine active infection. However, animals may be renal carriers of leptospira in the absence of active shedding, or the levels of leptospiruria may be too low to permit detection. Serologic screening for antibody to leptospira is recommended prior to translocation. Urinary identification of leptospira has poor sensitivity and will not be used to identify suitable candidates for translocation. The microagglutination procedure typically employs several antigens in a screening protocol. Due to the extensive serological cross-reactivity among the members of *L. interrogans*, screening with each of the more than 20 serovars is not required.

Salmonellosis

Salmonella infection has been described as endemic in Hawaiian monk seal populations in the wild and was isolated from five of seven seals captured on FFS in 1992 (Gilmartin, 1993). There may be substantial differences in pathogenicity among specific species within the bacterial genus. Salmonellosis is a gastrointestinal infectious disease which may result in death, especially in very young, elderly, and debilitated individuals. There are many examples of epizoodemics of salmonellosis with widespread morbidity and mortality, although such outbreaks are uncommon in marine mammals. *S. enteritidis* was associated with fur seal pup mortality on the Pribilof islands (Jellison, 1958), but not in fur seal or California sea lion pups on San Miguel Island, despite the isolation of the organism (Gilmartin, 1979). *Salmonella sieburg* and *Salmonella minnesota* were isolated from rectal cultures of monk seals (Gilmartin, 1980). Recent findings suggest that infection with *Salmonella* is prevalent among monk seal pups. Different *Salmonella* biotypes were isolated in 67% of seals transported for rehabilitation from FFS to Oahu from 1989-95 (NMFS unpubl. data, 1995). More recently, salmonellae were isolated from 9 of 12 (75%) weaned monk seal pups collected at FFS (Aguirre, 1998). The organism has been associated with mortality in monk seal pups undergoing rehabilitation in captive care settings. Further work defining the salmonella infection status of each of the outlying islands is recommended to establish the endemicity of the agent. Samples have been collected from Pearl and Hermes Reef, Midway, and FFS. Historical data on salmonellae infections at Midway Island also require analysis.

PROTOZOA

Toxoplasmosis

Toxoplasmosis, caused by the coccidian parasite *Toxoplasma gondii*, is an ubiquitous infection that has been described in marine mammals on several occasions. Fatal toxoplasmosis has been diagnosed in captive California sea lions (Migaki, 1977), in a fur seal (Holshuh, 1985), in bottlenose dolphins (Inskeep, 1990), in a spinner dolphin (Migaki, 1990) and in striped dolphins associated with morbillivirus infection (Domingo, 1992). Overwhelming toxoplasmosis often accompanies severe immunosuppression such as that caused by morbillivirus and will be considered a potential marker for that condition if it occurs in the monk seal. Toxoplasmosis is known to be endemic in rodents on small Pacific atolls that support feral cats that serve to complete the life cycle of the organism (Wallace, 1982). Toxoplasmosis could be transmitted to monk seals by dead rodents containing tissue cysts or by contamination of effluents with feral cat feces. A recent sero-epidemiologic survey testing for Toxoplasma gondii-specific IgG in harp seals, ringed seals, hooded seals, and minke whales from the North Atlantic was conducted (Oksanen, 1998): no positive samples were found.

APPENDIX III: GUIDELINES FOR TRANSLOCATION OF WILD HAWAIIAN MONK SEALS

Translocation and reintroduction of monk seals within the six subpopulation sites require careful planning, interdisciplinary participation, formulation of testable hypothesis and goals, thorough documentation, rapid publication of results, and review of the program by independent referees. The following considerations will be met prior to accomplishing a successful translocation plan (Wolff and Seal, 1993):

(1) Translocation studies are scientifically conducted management programs. Therefore, thorough reviews of literature and past attempts to translocate monk seals are necessary. There will be a protocol in place with all interdisciplinary parties within the program. Deviations from protocol will be approved by a preestablished process. The protocol will clearly state authority and responsibility (individual and organizational) for all aspects of the translocation. Data and documentation of all aspects are necessary: planning, financing, logistics, capture, transport, release techniques, results of post-release monitoring, and overall estimates of success. Sufficient funding will be allocated for completion of translocation and for post-release monitoring.

(2) A cost-benefit analysis will demonstrate that translocation is the most effective recovery strategy of the species with available funds.

(3) A baseline epidemiologic study is necessary prior to translocation.

(4) Translocation as used in this Epidemiology Plan will be primarily to reinforce a self-sustaining monk seal subpopulation. That is, no translocations will be allowed in declining or unstable subpopulations.

(5) Translocation will proceed only in ecologically suitable areas and appropriate habitat for the species. At this time, all six breeding subpopulation sites will be considered as recipient or donor populations.

(6) Translocation will proceed only where causes of threat to the population have been removed (i.e., human disturbance, predation, mobbing) and where there is minimal probability of serious pollution; human interaction; or exposure to predators, competitors, or disease.

(7) Translocation will proceed only when or where seal habitats are believed to be below carrying capacity, so as to avoid overcrowding and intraspecific aggression or excessive interspecific competition and predation.

(8) A thorough understanding of the monk seal's natural history, ecology, and behavior will provide guidelines to select the appropriate environment for translocation; determine appropriate numbers, social groupings, age, sex, and timing of reintroduction; predict mortality and reproductive levels; recognize disease and stress; and determine appropriate post-release management strategies.

(9) Seals for translocation will receive stringent veterinary examination and rigorous quarantine before they are moved into the recipient population. Reference blood specimens will be analyzed and banked. Individuals with evidence of disease or genetic defects or individuals with injuries, behavioral abnormalities, or other problems will not be translocated.

(10) In hard releases, the logistics required to move seals between sites must be carefully evaluated. For soft releases, it is necessary to confine seals for translocation in an enclosure or enclosures at the release site for several weeks for acclimation and health evaluation. This will provide quarantine for additional assessment before direct contact with the recipient population.

(11) There must be a long-term post-release monitoring effort which includes the recording of all disappearances, deaths, rescues and releases, births, and parentage. Causes of loss will be identified as specifically as possible. Monitoring should also include information on monk seal behavior and human interventions. Seals for translocation will be permanently identified for this post-release monitoring.

(12) Results of post-release monitoring will be used to adjust translocation protocols to improve survival and reproduction and to make the technique more cost-effective.

(13) There will be a contingency plan to discontinue or suspend the translocation if there is unanticipated mortality or other serious problem that has no immediate resolution.