Project Information

Project Title
Research and Enhancement of the Endangered Hawaiian Monk Seal, *Monachus schauinslandi*

Previous Federal permit #10137

Permits Requested
Permit for Scientific Purposes and to Enhance the Survival of the Hawaiian monk seal, under the Endangered Species Act, and for Scientific Research and to enhance the recovery of the Hawaiian monk seal under the Marine Mammal Protection Act.

Research and Enhancement Timeframe
1/1/2014 to 1/1/2019

Sampling Season/Project Duration
All aspects of field work may occur year round over the course of five years, with a peak of activities typically during March through September when Northwestern Hawaiian Islands (NWHI) field camps are typically deployed. Activities carried out in the NWHI during field camps (monitoring, photo-ID, bleach marking, tagging and retagging seals, measuring; disentanglement/collect marine debris; some translocations, aggressive male behavior mitigation; treating abscesses; conducting necropsies and collecting samples on the beach) would occur approximately from March through September annually, but may occur at any time of year. Timing of field camps ideally encompasses most of the pupping and nursing season, in order to monitor births and flipper tag all pups in each cohort. However, the timing of monitoring activities varies each year in the NWHI, driven in part by budget and logistics. The NWHI is an extremely remote location accessible by ship and by air to Midway (and French Frigate Shoals on a limited basis). All research and enhancement activities conducted in the Main Hawaiian Islands (MHI) would occur at any time of year.

Instrumentation in the NWHI would typically occur in the autumn – winter months, comprised of dedicated field efforts at a particular site annually (i.e., not necessarily during routine, long-term field camps). Health assessments are also conducted during dedicated efforts, and could occur at any time of year (especially in the event of an unusual mortality event). Antiparasitic treatments may occur year-round at specified intervals (i.e., summer, fall, winter, spring) with follow up assessments carried out between treatments. The first stage (weaned pup translocations) of two-stage translocation would typically occur in summer to autumn, whereas the second stage (returning juveniles seals), and experimental juvenile translocations could occur at any time of year. Vaccinations and supplemental feeding might also occur any time of year.

Abstract

This application seeks authorization to carry out research and enhancement activities designed to recover the endangered Hawaiian monk seal (*Monachus schauinslandi*). Research is intended to identify impediments to recovery, inform the design of conservation interventions and evaluate those measures. Enhancement activities are designed to improve the survival and reproductive

success of individual monk seals, with the intent to improve subpopulation and overall species' status.

Research activities include visual and photographic monitoring, tagging, pelage bleach marking, health screening, foraging studies, deworming research, experimental translocation, necropsies, tissue sampling, import/export of parts, behavioral modification research, and vaccination research.

Enhancement activities include deworming, translocation, hazing and removal of aggressive adult male seals that harm or kill other seals, disentangling, dehooking, behavioral modification, vaccination, and supplemental feeding of post-release rehabilitated seals.

Activities will occur along beaches and nearshore waters throughout the Hawaiian Archipelago [Northwestern Hawaiian Islands (NWHI) and main Hawaiian Islands (MHI)] and Johnston Atoll. Specimen samples may be imported/exported world-wide. The permit is requested for a 5-year period.

Annual number of animals to be taken by take type (annually, unless otherwise specified) would be 2,115 monitoring, 620 taggings, 35 sonic tagging, 1,495 bleach markings, 130 health screenings, 10 moribund seals by euthanasia, 60 instrumentations, 300 de-worming treatments, translocations of nursing pups to birth or foster mother as warranted (estimated 20 pups), translocations of weaned pups to alleviate risk as warranted (estimated 60 seals), 20 translocation of weaned pups and 30 juvenile/subadults as part of two-stage translocation for enhancement (**no seals would be moved from the NWHI to the MHI as part of two-stage translocation**), and 6 translocations of juveniles/subadults/adults for research, hazing aggressive adult males from conspecifics as warranted (estimated 10 seals), 20 adult male removals (including up to 10 lethal removals over five years), 10 captive adult males treated with testosterone reduction drug, unlimited (i.e., as warranted) disentanglements, dehookings, necropsies, opportunistic samplings and import/exports (including import and export of Mediterranean monk seal samples for research and conservation purposes), 12 seals supplementary fed, 20 seals subject to behavioral modification, 1,100 seals vaccinated, 400 incidentally harassed. Research on captive monk seals to test and validate field studies is proposed. The following lethal takes are annually/not to exceed in five years: 2/4 seals during research, 2/4 weaned pups during enhancement, 4/8 juveniles/subadults during enhancement, 2/4 adult males during enhancement.

Non-target species (non-listed) that occur in the study area and could be affected by the permitted activities include spinner dolphins (*Stenella longirostris*), bottlenose dolphins (*Tursipos truncatus*), and various nesting and/or brood-rearing seabirds that occur on or adjacent to beaches in the study area. Non-listed bird species that could be affected include Laysan albatross (*Phoebastria immutabilis*), black-footed albatross (*Phoebastria nigripes)*, wedge-tailed shearwater (*Puffinus pacificus*), masked (blue-faced) booby (*Sula dactylatra*), brown booby (*Sula leucogaster*), gray-backed tern (*Sterna lunata*), sooty tern (*Sterna fuscata*), black noddy (*Anous minutes*), brown noddy (*Anous stolidus*), and white (Fairy) tern (*Gygis alba*). The seabirds in this list all nest in beach areas frequented by monk seals, and the dolphins occur within the lagoon waters in the NWHI as well as in the main Hawaiian Islands (MHI).

Non-target ESA-listed bird species found in the NWHI that could be affected include: Laysan finch (*Telespiza cantans),* short-tailed albatross (*Phoebastria albatrus*), Laysan duck (*Anas laysanensis*), and Nihoa millerbird (*Acrocephalus familiaris kingi*). Also, green turtles (*Chelonia mydas*) basking on beaches may be affected by the permitted activities.

Project Purpose: Hypothesis/Objectives and Justification

General Statement Regarding Alternative Species: All of the research and enhancement activities described below are expressly directed toward the goal of recovering the endangered Hawaiian monk seal and are linked to the 2007 Recovery Plan for the Hawaiian Monk Seal (2007). Therefore, the proposed activities can only be conducted on the subject species, the Hawaiian monk seal, if they are to be informative and successful. That is not to say that when appropriate, relevant research on other species cannot inform the monk seal recovery effort. Indeed, there have been several cases where new methods or perceived risky procedures have been tested on surrogate species first. For example, seal-mounted video cameras (Crittercam) were used on several species prior to monk seals. Likewise, anti-helmintics (e.g., topical Profender) were tested on California sea lions before testing even on captive monk seals. And prospective vaccinations have and will be tested on other pinnipeds prior to monk seal trials.

General Statement Regarding Duplicative Efforts: NMFS is charged with research and recovery of the endangered Hawaiian monk seal, and the NMFS Pacific Islands Fisheries Science Center (PIFSC) Hawaiian Monk Seal Research Program (HMSRP) holds the only permit for research on the wild population (others hold permits for captive monk seals). Thus, research activities proposed herein cannot be duplicative as there are no other efforts to duplicate.

With regard to research proposed on captive monk seals, all of these seals are authorized to be maintained in captivity under separate permits issued to the holding facilities for enhancement and in all but one case, research purposes (Sea World, Sea Life Park, and Waikiki Aquarium) or researcher (Dr. Terrie Williams, University of California, Santa Cruz, Long Marine Laboratory). However, research proposed on these captive seals under this permit would not be conducted at facilities conducting the same studies (i.e., there would not be duplicated efforts on the same animals).

Some of the enhancement activities proposed herein (disentanglement, dehooking, and treating wounds) constitute response activities that may also be conducted under the auspices of the Marine Mammal Health and Stranding Response Program (MMHSRP). Authorization under the two different permits has proven to afford efficiency and flexibility in the past (e.g., in the event one party is not able to respond in a timely manner). The PIFSC HMSRP, PIRO (Pacific Islands Regional Office) and MMHSRP will continue to closely coordinate on all overlapping activities in the future.

General Statement Regarding Sample Sizes: Sample size planning is central to classical hypothesis testing research design. Ideally, sufficient replicates of an experiment or observational study are obtained in order to statistically detect a difference of a desired (hopefully biologically significant) magnitude given the level of variance in the parameter(s) being measured. For a variety of reasons outlined here, it is neither practical nor sometimes

desirable to strictly adhere to classical sample size determination for Hawaiian monk seal research and enhancement activities.

Low availability of samples—For most wildlife studies, the individual is the sample unit. Unfortunately, Hawaiian monk seals are rare and endangered, therefore few samples are available. In many, if not most, research projects undertaken with monk seals, it would be desirable to obtain larger samples sizes than are available or requested herein. For example, in the recent deworming trial conducted at Laysan Island (Gobush et al. 2011), every seal which met the age and condition criteria for inclusion was either a control or treatment subject in the study. The total of these groups was 43 seals and the results of the study (summarized in a later section) were equivocal. This may have been in part because of insufficient sample sizes. Nevertheless, a great deal was learned during the conduct of the study, which will shape future research and potentially enhancement efforts.

Balancing risks and benefits in setting samples sizes—Another key aspect of monk seal research and enhancement is that, because of the species poor status, a great deal of care is taken to minimize harm from any research activity. For this reason, requested samples sizes are lower than they might otherwise be if based solely on statistical grounds. For example, capture, sedation and instrumentation with telemetry devices entails some greater risk than other activities (photo-identification, etc.). As such, all adult females that appear or are likely to be pregnant are excluded from such studies. Past experience suggests monk seals may be subject to heightened risk around the time of molt, thus any molting or very recently molted seal is not captured. Further, seals compromised in other ways (severely wounded, emaciated, etc.) are excluded. These risk-averse decisions erode an already small available population from which to draw samples.

Related to the above, some activities are relatively novel and their risks not well-characterized. This is the case, for example, with experimental translocation of juvenile, subadult, or adult seals (described below). Thus, only up to 6 seals per year are requested. While this number may lead to uncertain conclusions, especially initially, it also avoids a catastrophic loss if the experiment were to fail with large sample sizes.

For both the above reasons: low availability of samples and balancing risks and benefits, the HMSRP has taken an approach of slowly accruing data over several years that will eventually yield statistically robust results, or if not, at least provide strong guidance for future research and enhancement.

Maximizing demographic data and enhancement benefits—Two final considerations have influenced the requested number of takes in this application. The long-term demographic database for Hawaiian monk seals is especially powerful for management because it allows for precise estimation of parameters. For example, simulation modeling (described below) is used to design and optimize prospective enhancement activities. The model used depends on complete abundance and age/sex structure information, which is obtained by attempting to totally enumerate all monk seal subpopulations. Therefore, regardless of classical statistical approaches, it is important to attempt to tag, bleach or photographically identify each animal in each subpopulation. Requested numbers of takes are designed to facilitate that goal.

Finally, the benefits of some enhancement activities, such as entanglement and dehooking, so clearly outweigh their risks, that unlimited numbers of such takes are requested.

RESEARCH OBJECTIVES and JUSTIFICATION **Population Assessment/Monitoring:** Primary objectives of population assessment activities are to evaluate the vital (survival and reproductive) rates and abundance trends of monk seal subpopulations, identify the threats to recovery, provide data that may be used to formulate recovery strategies for implementation, and evaluate the effectiveness of implemented recovery actions.

Extensive population assessment activities are carried out at six NWHI subpopulations, where field camps typically up to five months in duration are deployed annually. Less frequent surveys are also conducted at Necker and Nihoa Islands (NWHI), and information is gathered, largely through a volunteer sightings network, year round in the MHI. Aerial surveys (including unmanned aerial vehicles), boat surveys, unmanned terrestrial or amphibious vehicles, and remote video cameras complement ground-based surveys. The foundation of the population assessment program is long-term marking and resighting of individual seals.

Objectives at each site include:

- Conduct regular systematic surveys to count and identify all individuals on the beach at any given time ("beach counts")
- Enumerate (through individual identification), to the degree possible, all the seals occurring at each subpopulation
- Tag and measure pups and other seals for permanent identification and monitoring body condition, and retagging animals to maintain identification
- Obtain digital photographs to maintain an updated photographic identification database
- Collect tissue for genetic analysis
- Document births, identify parturient mothers and determine duration of nursing period
- Document any factors that may affect the survival of individual seals, including wounds (from sharks, aggressive males), entanglement in marine debris, hookings, emaciation, etc.
- Document any deaths or disappearances of seals
- Document "association" data that may be used to identify aggressive male seals that cause injury or death to other seals.

The Hawaiian monk seal is the subject of a long-term (approximately 30 yr), consistent and very detailed demographic study (Bowen et al. 2010). This study has been integral to every aspect of Hawaiian monk seal conservation and management for several decades. Annual field data are entered into a relational database, from which output is analyzed to estimate the following parameters:

- Subpopulation abundance
- Subpopulation age-sex composition
- Age-specific survival
- Gross and age-specific reproductive rates
- Movement rates among subpopulations.

In addition to these standardized analyses, information on frequencies of causes of injury and death are tracked in order to identify threats to recovery. Also, genetic analysis of population and individuals is conducted.

The information above has been used to closely track that status and trends of the species, identify threats and evaluate recovery actions. They have also been used to evaluate the potential negative effects of research activities.

Another objective of population assessment is to provide the basis for a better understanding of Hawaiian monk seal ecology. Thus, trends in parameters such as survival, body condition, and abundance are analyzed to establish links with climate-ocean dynamics in order to better understand the drivers of monk seal population trends.

One of the most important objectives of population assessment is to feed current data into a purpose-designed Hawaiian monk seal stochastic simulation model (Harting 2002). This model is used to synthesize demographic information, project future trends, and most importantly, evaluate the potential efficacy of conservation interventions and also evaluate the impacts of current or potential threats on population recovery.

Annual monitoring of NWHI monk seal subpopulations, including all the attendant data gathering activities as described, has been identified in the Hawaiian Monk Seal Recovery Plan (NMFS, 2007) as a Priority 1[1] research action. Annual monitoring in the NWHI and aerial surveys in the MHI have also been recommended by the Marine Mammal Commission (2002). A series of external reviews of the HMSRP (Bowen, 2001; Siniff, 2001) also recommended continuation of annual monitoring of Hawaiian monk seal populations.

There is an extensive body of literature documenting analyses of Hawaiian monk seal populations. Key and recent publications are listed in Table 1.

Table 1. Selected key and recent Hawaiian monk seal population assessment publications, arranged by topic or category. The Draft Programmatic Environmental Assessment for Hawaiian monk seal Recovery Actions, which serves as the draft National Environmental Policy Act (NEPA) documentation for this application, provides information on the following topics. The Draft PEIS is available at: http://www.nmfs.noaa.gov/pr/permits/eis/hawaiianmonksealeis.htm.

Topic or Category	**References**
Abundance and abundance trends	Baker 2004, Baker and Johanos 2004, Baker et al. 2006, Carretta et al. 2009
Survival	Baker and Thompson 2007
Reproduction	Harting et al., 2007, Johanos et al. 1994
Ecological relationships	Craig and Ragen 1999, Antonelis et al. 2003, Baker et al. 2007, Baker 2008
Simulation Modeling	Harting 2002, Baker et al. 2010

[1] "An action that must be taken to prevent extinction or to prevent the species from declining irreversibly."

Topic or Category	References
Handling effects	Henderson and Johanos 1988, Baker and Johanos 2002
Threats and mitigation	Hiruki et al. 1993a, 1993b, Donohue et al 2001, 2007, Henderson 2001, Johanos et al. 2010,
Photographic Identification	Harting et al. 2004
Genetics	Schultz et al. 2009, 2010, 2011a, 2011b

Marking (flipper, PIT, and sonic tags; bleach marks) Objectives and Justification:
Flipper tags (e.g., Temple, Roto and monel tags): Researchers apply a variety of marks to facilitate both short- and long-term identification of individual seals, which is the most critical foundation of the population monitoring database. The most commonly applied marks are lettered and numbered flipper tags. Flipper tags are applied to both hind flippers of weaned pups and to older individuals that may not have been tagged previously. Tags would be re-applied to individual seals whose tags have become lost, broken, or excessively worn, in order to maintain the individual identities of these animals. Temple tags are typically applied to weaned pups and older seals, however other tags (e.g., Roto or monel tags) may be applied when standard capture and restraint is not possible or warranted (e.g., pre-weaned pups).

PIT tags: After flipper tags have been applied, but while the seal is still under restraint, a Passive Integrated Transponder (PIT) tag is typically injected. These are the same kind of "chip" commonly inserted in domestic dogs and cats to facilitate unique identification. The unique identifying code of each chip can later be determined using portable, hand-held readers, thereby providing long-term maintenance of identity even if flipper tags are lost.

Flipper tagging nursing pups: Monk seal field research camps currently do not typically span the entire pup-nursing season, and researchers often depart camps when some pups are still nursing. The fate and identity of these pups is usually unknown because they are not tagged prior to weaning. Tagging as many pups as possible is critical to demographic and other analyses. Individuals first tagged as pups are used to compute age-specific vital rates (survival and reproductive rates) because their ages are known. Also, it is impossible to associate the identities of unmarked juveniles with individual pups born in the previous year, so that failure to tag each pup reduces the ability to track the fate of pups born at particular islets or to individual females. These concerns, when compounded over time, may severely reduce our understanding of the life history dynamics for this population.

In order to better monitor the fates of these seals and obtain larger and more representative samples of each cohort, we propose to tag pre-weaned pups using methods that minimize disturbance. Currently, pups are not tagged until they have weaned so as not to disturb mother-pup pairs. However, nursing pups are frequently bleach-marked, which can be accomplished with little or no disturbance. These marks are not visible for more than a few weeks unless they are applied near the end of the nursing period when pups have already molted. Here, we propose to apply a single tag using minimal restraint (e.g., a Roto or monel tag) to some pre-weaned pups. This would only occur in cases where pups have not and will not wean prior to the end of field camps (otherwise, researchers would wait until weaning as usual to apply tags). Surviving pups may be recaptured as yearlings (the subsequent year) and retagged with the longer-lasting Temple tags.

Sonic tags: A limited number of weaned pups may also be marked with a small sonic tag. Galapagos shark predation at French Frigate Shoals has drastically decreased pup survival for more than a decade. The primary purpose of sonic tagging is to gain information to aid in reducing this predation on weaned pups. Movements of pups and proximity to sonic-tagged sharks for the time period just after weaning is monitored via sonic tags attached to flipper tags. Receiving stations "listen" for both shark and seal sonic tags and record them when they are in range. These data are used to better inform management actions aimed at reducing shark predation, such as culling sharks.

Bleach marks: Bleach marking seals' pelage (fur) is another integral part of individual monk seal identification. Most of the seals to which marks are applied have been previously tagged and have an identity assigned. The presence of a highly visible bleach mark facilitates re-identification of an individual from a much greater distance than would otherwise be the case if researchers relied on flipper tags alone. Thus, there is less need to approach bleached seals closely, thereby reducing disturbance.

Marking monk seals directly supports annual monitoring of monk seal subpopulations, which has been identified in the Hawaiian Monk Seal Recovery Plan (NMFS, 2007) as a Priority 1[2] research action.

Collect morphometric and ultrasound measurements to determine body condition – Objectives and Justification: Measurements of axillary girth and dorsal straight length are indicators of Hawaiian monk seal health and body condition (Craig and Ragen 1999). These data have proven especially useful for comparing condition of seals in different subpopulations and provide insight into the factors that affect survival and population trends. Seals are also sometimes weighed and blubber depth measurements are sometimes collected using a portable imaging ultrasound. Blubber depth measurements indicate condition and nutritional state by assessing fat stores in the body.

Health Studies – Objectives and Justification: A variety of research activities focus on monk seal health. These studies all address one or more of the following objectives:

- Baseline health assessment to establish normal values for morphometric, hematologic and biochemical parameters within age and gender classes
- Monitor hormones to assess individual condition and reproduction
- Monitor and document infectious disease to help understand its influence on past, current and potential future population trends
- Develop prevention and control strategies to mitigate the effects of suboptimal health
- Develop contingency plans for high risk unforeseen circumstances

[2] "An action that must be taken to prevent extinction or to prevent the species from declining irreversibly."

- Provide guidance and protocols where there is a nexus between health issues and enhancement activities (e.g., translocations between subpopulations).

Baseline health assessments – Current knowledge of infectious diseases impacting Hawaiian monk seals is based on results of epidemiological surveys of live animals sampled during die-offs (Gilmartin et al. 1980; Aguirre et al. 1999; Aguirre 2000), necropsy examinations of individual dead monk seals (Gilmartin et al. 1980; Banish and Gilmartin 1992), and sampling of overtly healthy animals (Aguirre et al. 2007; Littnan et al. 2006). The purpose of the proposed research is to continue to collect data on pathogens that might impact Hawaiian monk seals, either individually or as a zoonotic or unusual mortality event (UME). Screening of debilitated seals would be to diagnose disease or other conditions which may be affecting an individual seal, or, in the event of a UME, a large number of seals. Screening of healthy seals would be done selectively to help gain a basic understanding of disease exposure to the population, and to establish normal baseline values for hematologic and biochemical parameters.

Hormones –Hormone measures may provide novel, sensitive information about individual condition and reproduction in Hawaiian monk seals. For example measures of glucocorticoids, thyroid hormone (triiodothyronine), and reproductive steroid hormones from free-ranging wildlife have been used to monitor individual response to disturbance, changes in metabolism during food shortages, and reproductive activity. Glucocorticoids (including cortisol), also known as stress hormones, increase in circulation and feces in response to psychological and nutritional stress. Thyroid hormone has profound influences on metabolism, heart rate, blood pressure, nutritional physiology, and body temperature regulation independent of muscle activity. Thyroid hormone is particularly responsive to nutritional deficits and decreases in response to acute and chronic starvation, lowering metabolism and making the body conserve energy during a nutritional emergency. Progesterone measures are frequently used to detect pregnancy and its progression, while testosterone measures can indicate an active reproductive state in males.

All of these hormones have been measured in Hawaiian monk seal serum in the past, and validated and analyzed to different degrees (testosterone: Atkinson 1993, cortisol and thyroid hormone: Atkinson & Oki *unpubl.*, progesterone: measured but not analyzed, Braun *pers comm.*). Fecal progesterone and testosterone measures were validated and analyzed using the current methodology of the time (Kirby 1995). Fecal glucocorticoid and thyroid hormone measures were functionally validated recently and covariates analyzed (Gobush et al. *in preparation*).

Recent advances in hair analysis demonstrate that hormones such as cortisol and progesterone can also be found in keratinous tissues (Macbeth et al. 2010, Gow et al 2010). Analysis of whisker sections for cortisol could then identify seasonal changes in stress (Mostl and Palme 2002); and analysis for progesterone could identify pregnancy via elevated progesterone; and potentially shed some light upon pup survival. The HMSRP has good records on most pregnancies being carried to term, however, there are a number of seals that appear not pregnant or fail to carry a pup to term. If a seal is pregnant but aborts, or the pup was lost, the female would go into estrus, which is accompanied by elevated progesterone. By examining the

whisker sections for progesterone, the lack of a section with depressed progesterone would indicate that lactation did not occur and suggest pregnancy termination or pup loss. This type of information could help with better understanding the apparently low reproductive rate of the monk seal population and see if there may be underlying factors (such as brucella) that are reducing the number of females seals carrying pups to term.

Going forward, all of these hormones and potentially others may be extracted from monk seal serum, fecal, or vibrissae samples from any age class, gender or location for physiological monitoring. Captive animals may be sampled for hormone analysis. Procedures for storage, hormone extraction and laboratory analysis will be standardized for samples of each type (serum and feces) to allow for comparison of measures across future studies.

Disease studies—Through a variety of methods described in a later section, exposure, incidence, and prevalence of infectious disease are monitored in the various Hawaiian monk seal subpopulations. Current information suggests infectious disease is not limiting recovery of the Hawaiian monk seal (NMFS 2007). However, the species is rare, has very low genetic diversity and may have been buffered from exposure to many mammalian diseases due to its isolation in the Hawaiian Archipelago for millions of years. Together, these factors raise great concern that outbreaks of diseases to which monk seals have not been previously exposed could have devastating impacts (NMFS 2007).

Certain pathogens are of special concern (West Nile virus, morbillivirus) in that they have not yet been detected in wild monk seals but could have devastating effects on the species should outbreaks occur. There is especially heightened concern about the risk of new disease exposure in the MHI where monk seals encounter land mammals (and, potentially their diseases) to which they have not been exposed during their long isolated evolutionary history in Hawaii. One of the enhancement activities proposed below involves translocation of seals between subpopulations. Proper population-level surveillance as well as individual screenings will be required to ensure that spread of disease is not facilitated by human interventions. The Recovery Plan lists "*Reduce the probability of the introduction of infectious diseases into the Hawaiian monk seal population*" as one of the "*four key actions required to alter the trajectory of the Hawaiian monk seal population and to move the species towards recovery*" (NMFS 2007). Clearly, disease research is central to this goal.

An Unusual Mortality Event (UME) contingency plan has been published by the HMSRP for the monk seal (Yochem et al., 2004). Protocols have been developed for a variety of procedures including sample collection and banking, and necropsy examinations, and training has been instituted for field staff. Archives of tissues and samples have been developed by sampling all animals sedated for research purposes and by performing complete necropsies on all dead animals found. Cell cultures of skin, brain, lung, kidney and spleen have been established in laboratories for potential future analysis and isolation of pathogens. Cooperating agencies, including the facility maintaining a cell line, are listed under "*agencies/cooperators*" below.

Further efforts are required to reduce infectious disease outbreak risks. The proposed research includes vaccination studies to determine the safety and efficacy of vaccines against specific pathogens considered most likely to spread to monk seals (for example, Morbillivirus and West

Nile Virus). Captive studies would include both monk seals and surrogate species, and potentially free-ranging Hawaiian monk seals. If such research indicates that such vaccines are safe and effective, they may be administered preventatively or in response to an outbreak (as an enhancement activity).

Deworming—The following is largely excerpted from a manuscript regarding deworming research conducted to date (Gobush et al. 2011).

Hawaiian monk seal abundance is declining due to low juvenile survival, which appears to be associated with food limitation and poor body condition (Baker 2008). Parasites compete with their host for nutrition and parasite burden is associated with decreased growth, immune competency and survivorship in pinnipeds and other species (DeLong et al. 2009). Hawaiian monk seals are known to host a variety of gastrointestinal parasites (Dailey et al. 1988, 2004). Thus, parasitic infection may play an influential role in the maintenance of condition, especially when food is limiting and a seal's immune system is developing. Reducing the gastrointestinal helminth burdens in domestic livestock with anti-helminth medications is a common veterinary practice and shows promise as an intervening measure to improve juvenile monk seal condition as well.

Two families of cestodes (i.e., tapeworms), *Diphyllobothriidae* and *Tetrabothriidae*, and two families of nematodes (i.e., roundworms), *Ancylostomatidae* and *Anisakidae* infect the gastrointestinal tract of pinnipeds (Dierauf & Gulland, 2001). Pathogenic effects have been associated with both cestode and nematode infection in pinnipeds (Dierauf & Gulland, 2001). However, the more frequent, insidious and long-term effect may be the parasites' competition with the host for nutritional resources and the immune response they may elicit in the host. Reif et al. (2006) reported that young seals at French Frigate infected with Diphyllobothrium species (tape worms) tended to be in poorer body condition than those uninfected. Cestodicidal pesticide Praziquantel (a de-worming drug) is widely used to control parasites of domestic animals and livestock and the drug is a commonly used prophylactic for the treatment of rehabilitated pinnipeds (Andrews & Thomas, 1983, Dierauf & Gulland 2001). Anecdotally, some captive monk seals have passed tapeworms within days of Praziquantel treatment (NMFS, unpublished data).

In 2009-2010, the HMSRP evaluated the efficacy of anti-helminth treatment on Hawaiian monk seals and the utility of this intervention for improving juvenile monk seal survival. The following hypotheses were tested:
1) a common deworming medication, injectable Praziquantel, is safe and effective in reducing worm burdens in juvenile monk seals, and
2) intermittently reducing worm burdens in juvenile monk seals is associated with better body condition and increased probability of survivorship.

The results of this study were equivocal. A total of 43 juvenile seals at Laysan Island were either serially treated with intramuscularly (IM) injected Praziquantel or served as controls. Both groups experience high survival which did not different between groups. Percent mass gain was significantly greater for treated than control seals during March to May, but not during December to March or over the entire treatment period (December to May). An efficacy trial

indicated that IM Praziquantel may not be effective in treating cestode infection in monk seals. Questionable feasibility and weak efficacy indicate that a different route of administration or dosage of Praziquantel or a different anti-helmintic may be more suitable for this species in the wild. A small pilot study found that it was not feasible to accurately administer oral dewormers to monk seals in a remote field setting.

The proposed research would continue to seek effective means for treating parasitic infections in free-ranging monk seals and to determine if this treatment can help improve survival and body condition. If so, this could be a tool implemented for enhancement under the proposed permit (see below).

The proposed activities have been recommended as priority 2 research and intervention actions in the Hawaiian monk seal recovery plan (NMFS, 2007). Priority 2 action 4.1.7. states "Investigate whether controlled research on deworming could be conducted (on other species or on monk seals) in order to improve juvenile survival by reduction of parasite stress, including the potential negative impacts if not conducted properly."

Contaminant studies— Persistent organic pollutants (POPs) originate from anthropogenic substances such as pesticides, industrial chemicals, flame retardants, or occur as chemical byproducts (Bard et al. 1999). Although many POPs have been banned from use in North America and Western Europe, some nations still use these substances. POPs are persistent in the environment due to their long half-lives and resistance to degradation. POPs are lipophilic and tend to accumulate in the blubber and other fatty tissues of animals.

Contaminants are often measured in blubber, liver, and blood of animals because these are tissues in which the contaminants concentrate or which are relatively easy to obtain from live animals. Hawaiian monk seals, like other mammals, accumulate POPs such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), and polybrominated diphenyl ethers (PBDEs) in their tissues through nursing when young and through their diet later in life.

Two studies have quantified POPs in Hawaiian monk seal tissue but none have yet assessed effects of these compounds on the seals. The first study investigated PCB and DDT levels in the serum and blubber of 46 individual seals from French Frigate Shoals (Wilcox et al. 2004). The presence and levels of 14 PCB congeners, DDT and DDT metabolites was examined. This study found patterns in contaminant level associated with the sex and age-class of the seals. Adult males had significantly higher PCB levels than reproductive adult females and immature seals of both sexes. Only one DDT metabolite (p,p'-DDE) was detected in the blubber, and none in any serum samples. Age, sex, reproductive history, and minimum number of pups were not significantly correlated with PCB levels in the blood or blubber (Wilcox et al., 2004).

The second study investigated contaminant levels in whole blood and blubber of 158 individual seals from four NWHI populations (French Frigate Shoals, Laysan Island, Pearl and Hermes Reef, and Midway Atoll). This study also found patterns in contaminant levels relating to life history traits of the seals. Adult males and juveniles from Midway Atoll were found to have higher total PCB levels compared to individuals of the same age and sex from the three other

NWHI sites tested (Ylitalo et al. 2008). A recent study characterized contaminant levels in Hawaiian monk seals in the MHI (Lopez et al. 2012).

Multiple studies have shown links between contaminant exposure and detrimental health effects such as reproductive impairment, immune dysfunction, and cancer in several pinniped species (northern fur seals: Beckmen et al. 2003, harbor seals: De Swart et al. 1994, California sea lions: Ylitalo et al. 2005a and DeLong et al. 1973). Although contaminant exposure is often discussed as a correlate to these sub-lethal effects, a causative relationship can be difficult to determine without experimental data. Of the studies above in which contaminant effects (or correlations with contaminant levels) were detected, only the Ylitalo (2005) study was comparable (in terms of tissue, age class, and units measured) to the monk seal studies. Summed PCB and DDT levels were approximately one or two orders of magnitude higher in the California sea lions Ylitalo (2005) analyzed compared to the contaminant levels measured in the two NWHI monk seals studies.

The proposed actions are to continue collection of samples for contaminant analysis to monitor for changes and better characterize the potential influence of these substances on monk seal health and recovery.

Contaminants were considered a moderate threat in the Hawaiian Monk Seal Recovery Plan, and the following two recommendations were included:

"11.1 Continue collection of samples from seal and prey species and banking of samples for potential contaminant monitoring

11.2 Examine data for association between reproductive failure and exposure to contaminants and conduct a risk assessment specific for monk seals"(NMFS 2007).

Necropsy—The HMSRP recognizes the value of examining dead Hawaiian monk seals, and has routinely collected samples from carcasses at every opportunity. Dead seals provide additional information on the health and ecology of the species. Examination of tissue samples can reveal illnesses which afflicted the seal, and perhaps the cause of the animal's death. Determination of parasite load provides information on the overall health of the animal, and examination of stomach contents can help determine food habits and foraging behavior. Samples of muscle, organs, or blubber can be examined for presence of environmental contaminants. Dietary information can also be obtained from necropsies through examination of gastrointestinal contents, blubber samples for fatty acid analysis, and a variety of tissues for stable isotope analysis. Skeletal provide valuable reference materials.

Necropsies have been identified in the Hawaiian Monk Seal Recovery Plan (NMFS, 2007) as a Priority 1 protection action. Specific actions are: 4.3 "Maintain current disease monitoring programs".

Table 2. Selected key and recent Hawaiian monk seal health-related publications, arranged by topic or category.

Topic or Category	**References**
Infectious Disease	Banish and Gilmartin 1992, Aguirre 2000, Aguirre et al. 1999, Nielson et al. 2005, Littnan et al. 2006, Aguirre et al. 2007, Goldstein et al. 2009,
Parasites	Dailey et al. 1988, Reif et al. 2006
Baseline parameters	Reif et al. 2004
Contaminants	Willcox et al. 2004, Ylitalo et al. 2008
Ocular disease	Hanson et al. 2009
Contingency planning	Yochem et al. 2004

Foraging Studies – Objectives and Justification: Foraging success of juvenile Hawaiian monk seals is a key element affecting demographic vitality of this highly endangered species. Continued declines in the abundance of monk seals at several colonies and poor recovery at others has been attributed to poor survival and low recruitment of juveniles owing primarily to their relatively poor foraging success. However, other age/sex classes also demonstrate the effects of poor foraging success. A Priority 1 recommended action in the Recovery Plan is to "*Define diet by age, sex, location, season (variety of methods) and characterize feeding areas quantitatively.* Acquisition of those data are needed to determine the functional relationships among foraging effort and foraging success of pups, juveniles, and adults and survival, recruitment, fecundity, and population status.

Telemetry studies—Monk seal dive behavior, geographic dispersion and habitat use will be investigated by testing specific hypotheses with enhanced accuracy of the information collected. Example hypotheses include:

- Foraging patterns of NWHI and MHI monk seals vary consistently with differences in demography and body condition.

- Monk seal foraging habitat use in the MHI overlaps spatially with commercial, recreational and subsistence fishing

- Foraging behavior of juvenile monk seals in the NWHI is correlated with variable oceanographic conditions

Additionally, research will focus on better characterization of the nature, extent, frequency and spatial dynamics of monk seal interactions with fishers and fishing in the MHI.

Improved characterization of geographic and vertical habitat use of monk seals are now possible by integrating telemetry instruments that provide detailed information on diving patterns (i.e., vertical foraging habitat) with fine scale information on geographic habitat use to allow direct temporal and spatial matching of three dimensional habitat use. That fine scale integration is important for determining how foraging effort and foraging success of seals may by influenced by the foraging patterns and interactions with conspecifics and other competitors.

Many monk seals forage in complex habitats or in regions where habitat (depth, substrate, etc.) can change substantially over short distances. Proposed research will incorporate technologies (e.g., Global Positioning System or GPS) that can provide fine scale spatial resolution (i.e., within meters).

The number of animals to be used is determined in large part by the number of animals of suitable age available at a particular site. Existing data on Hawaiian monk seal foraging ecology indicate high variability in depth, duration, and location of dives. Due to high individual variability and the small sample sizes involved in these studies, appropriate non-parametric techniques will be used to compare differences or changes in monk seal foraging behavior over space and time.

Diet studies— Monk seals forage mostly in demersal and benthic habitats along the Hawaiian submarine ridge and to depths of 500 m or more. Their diet includes a variety of fishes, cephalopods, and crustaceans (Rice, 1964; MacDonald, 1982; Goodman-Lowe, 1998; Parrish et al., 2000; Parrish et al., 2002; Longenecker et al., 2006). Goodman-Lowe (1998) found that fishes comprised the majority (78.6%) of monk seal diet, followed by cephalopods (15.7%), and crustaceans (5.7%), using analyses of prey hard parts recovered from seal feces (scats) and vomitus (spews). Of the thirty-one families of fishes identified, the most common were marine eels (a group composed of morays, congers and snake eels), followed by wrasses, squirrelfish and soldierfish, triggerfish, and parrotfish. Cephalopod prey included seven species of octopus and 19 species of squid. Monk seals forage in both shallow and deeper water habitats (>300 m) in precious coral beds and on the tops of submerged seamounts and banks in the subphotic zone, which are habitats for some species (e.g., eels) that are known monk seal prey (Parrish et al., 2002; Longenecker et al., 2006).

In a study using Quantitative Fatty Acid Signature Analysis (QFASA) across multiple locations in the NWHI and MHI, monk seal diets were estimated to be comprised of a mixture of species. The most abundant estimated prey species were boarfish, duckbill, box crab, flower snapper, shrimp, squid, squirrelfish snapper, and tang/surgeonfish. Overall, estimated diets of monk seals during the period of this study (1998-2002) were dominated in large part by deep-slope species, consistent with recent results from seal dive and location analyses, as well as from animal-borne video. However, there was substantial variation in diet among individuals, demographic groups (especially between juveniles and adults/subadults) and locations (Iverson et al. 2011).

To look at long-term foraging patterns, the HMSRP proposes a study to identify temporal changes in diet via analysis of stable isotope ratios obtained from serial sections of monk seal whiskers (vibrissae). Previous studies indicate that phocid whiskers grow continuously and shed annually (Greaves et al 2004). Because whiskers are metabolically inert, each section represents

diet during the period of growth (Hirons et al 2001, Kurle and Worthy 2002). Stable isotope ratios calculated from different sections of the whiskers can then be used to interpret the diet or trophic position of the seals (Hobson and Welch 1992, Hobson et al. 1996, Zhao and Schell 2004).

Use of an alternative, non-listed species is not appropriate to study the diet of Hawaiian monk seals. Investigating and mitigating factors affecting food limitation of Hawaiian monk seals has been identified as a Priority 1 research activity (Action 1) of the Hawaiian monk seal recovery plan (NMFS, 2007).

Although much has been learned in the past ten years about monk seal diets, many important questions remain that, if addressed, would improve conservation efforts for this species. For example, the growing monk seal population in the MHI is perceived by many fishers to be impacting catch of certain desired species. The degree of overlap between monk seal diets and those species targeted by fishers remains unclear. Drivers of variable prey consumption (climate variability, oceanic productivity, inter- and intra-specific competition) must be better understood to inform spatial and temporal decisions about monk seal translocations and other enhancement and management decisions.

Opportunistic Sample Collection—This activity primarily supports diet analysis but also has some health-related applications. The objective of the proposed activity is to gather specimens which can provide important biological information but which nonetheless will not directly take animals. This activity is intended to gather maximum biological information from Hawaiian monk seals, so use of an alternative species is not appropriate. Fecal material (scats) and vomitus (spews) collected from beaches are a primary source of dietary information. Shed molt samples found on the beach provide genetic material, and placentae may provide information for health and disease analysis.

The proposed actions will continue telemetry and diet studies using the latest technology available and a suite of methods (hard parts analysis, fecal DNA, fatty acids and stable isotopes).

Table 3. Selected key and recent Hawaiian monk seal population foraging publications, arranged by topic or category.

Topic or Category	**References**
Habitat use	Littnan et al. 2006; Parrish et al. 2000, 2002, 2005; Stewart et al. 2006
Competition	Parrish et al. 2008
Diet	Goodman-Lowe 1998, Longenecker 2010, Cahoon 2011, Iverson et al. 2011,
Research effects	Littnan et al. 2006

ENHANCEMENT OBJECTIVES and JUSTIFICATION

Translocation: According to the "IUCN Guidelines for Reintroduction", translocation is defined as "deliberate and mediated movement of wild individuals or populations from one part of their range to another" (IUCN 1998). Translocation has proven to be one of several useful tools in the Hawaiian monk seal conservation effort. Baker et al. 2011 provide a review of past Hawaiian monk seal translocations. The following is excerpted from that publication's abstract:

> Over the past three decades, numerous monk seal translocations have been conducted with a variety of objectives, including mitigating shark predation and conspecific male aggression, reducing human–seal interactions, and taking advantage of favorable foraging habitats to improve survival. Here, we analyze our cumulative experience with translocation of Hawaiian monk seals. We found a strong correlation between the time seals remained in the vicinity of the release site and their age. Recently weaned pups (with little or no at-sea foraging experience) exhibited high fidelity to release sites commensurate with that shown by untranslocated pups to their birth location. In contrast, juvenile and adult seals tended to stray from their release locations farther and sooner. Nevertheless, when 21 adult male seals were moved more than 1000 km from Laysan Island in the Northwestern Hawaiian Islands (NWHI), to the main Hawaiian Islands (MHI), they subsequently dispersed among the MHI; however, only one was observed to return to the NWHI. Translocated seals appeared to survive at rates comparable to seals native to the release site. Outcomes suggest that in most cases the intended objectives of translocations were achieved. Except for one notable case, translocations within the MHI to arrest human–seal interactions were mostly unsuccessful.

The following translocation actions are proposed to address a variety of threats to monk seal survival:

Nursing, or pre-weaned pups separated from their mothers may be captured, and relocated to a prospective foster mother or back to their natural mother, respectively. Young pups that are prematurely weaned or otherwise separated from their mother suffer high rates of mortality. In these cases, intervention to restore nursing can enhance the pup's survival. Pup switching, whereby two adult females exchange the pups they are nursing, either temporarily or for the duration of lactation, is relatively common in monk seals. Sometimes these situations compromise the survival of one of the pups. For example, if a young pup is switched to a mother who has nearly completed her lactation period, that pup will wean prematurely and likely starve. In related cases, a previously weaned pup may displace a young pup from its mother or nurse alongside the pre-weaned pup. In cases where the disadvantaged pup is a female and the other pup is a male, field researchers may remove the male pup to a safe distance and reunite the female pup with its mother. During these handling events, pre-weaned pups may also be tagged.

Weaned (or nearly weaned) pups in locations where there is a severely reduced chance of survival, such as areas of high shark predation (e.g., some islets at French Frigate Shoals), disease or contaminant exposure, or likelihood of human interaction (for example, hooking, entanglement, socialization, disturbance in the MHI), may be moved to locations which present

less risk. In such cases, pups born within the NWHI are moved to other sites within the same NWHI atoll, and pups born within the MHI are moved to other beaches or islands in the MHI.

Seals of any size and either sex occasionally find themselves in high risk locations. For example, pups sometimes become trapped behind a sea wall on Tern Island and seals occasionally haul out on boat ramps or near roads in the MHI. In such cases, these seals may be carefully hazed (or if need be, capture and carried) out of the dangerous area in order to alleviate the risk of harm or death.

Weaned pups and juvenile seals in subpopulations where juvenile survival is low may be translocated to subpopulations with higher rates of juvenile survival. Survival at the original site may be relatively low due to insufficient prey availability (thought to be the primary cause of juvenile mortality), but may also be affected by other factors. The current Permit No. 10137-07 allows for such translocations only among subpopulations *within* the NWHI. Here, we seek to apply this tool to also move seals from the MHI to the NWHI when deemed warranted. Also, this application seeks permission for a return translocation of individuals back to their natal or other suitable subpopulations once they have reached an age when their survival probability is universally quite high.

Details on this approach, referred to as *two-stage translocation*, can be found in Appendix A. **This appendix presents scenarios that include translocating weaned pups from the NWHI to the MHI for demonstration purposes only. The principles and concepts of two-stage translocation are consistent regardless of which specific source and host sites are involved. Permission to translocate weaned pups from the NWHI to the MHI may be requested in five years, but will not be conducted prior to that time.**

Experimental translocation of seals age 2-3 yr and older is proposed (as research) to better anticipate benefits of two-stage translocation. Under two-stage translocation, the earliest data about the actual return survival decrement would not be available until the fourth year of the project, when the survival of the first group of 2-3-yr old seals returned to their natal sites would be evaluated. Relevant information could, however, be collected by initiating some limited experimental translocation of juvenile seals.

The experiment may first involve moving a small number of juveniles or subadults among areas of the NWHI where foraging conditions or success are thought to be comparable. This would help evaluate the potential combined effects of translocation on this age-class, without the confounding influence of a marked change in habitat quality. Subsequently, juveniles or subadults might then be moved from an area with relatively low competition and predator densities (e.g., the MHI at present) to areas with greater competition and higher predator densities (NWHI). This would provide information about how older juveniles respond to being released in unfamiliar environments with more challenging conditions relative to where they grew up.

Seals with unmanageable human interactions could be taken from the MHI to the NWHI. Occasionally, individual seals in the MHI develop habitual patterns of seeking out humans and interacting with them, sometimes in ways that constitute a risk to the individual seal as well as a

public safety risk. Research to develop tools to prevent and mitigate human interactions with individual seals is proposed (see below, Behavioral Modification). However, there are likely to be cases in the future, as there have been in the past, where despite all efforts to alter seal or human behavior, the interactions persist. In such cases, unmanageable seals could be translocated from the MHI to the NWHI, where they could continue to live in a wild population that is isolated from human contact. In past cases, such seals typically involved permanent captivity or translocation to Johnston Atoll where there are only occasionally other seals. Those solutions resolve the public safety concerns but do not benefit the individual monk seal or any seal population. Translocation to the NWHI would constitute enhancement as the seals would have an opportunity to continue living wild and contribute to recovery.

Aggressive male monk seals may be translocated as one option to mitigate their effects on other seals. This will be discussed in the following section.

Translocations are intended to directly enhance the survival of individual Hawaiian monk seals, so use of an alternative species is not appropriate. Direct benefit will accrue to those animals that are translocated, or in the case of aggressive male translocations, to seals no longer exposed to the translocated males. Translocations of seals to protect them from predation has been identified as a Priority 1 intervention action and enhancing survival by translocating juvenile female seals to areas of higher survival probability has been identified as a Priority 2 intervention action in the Hawaiian monk seal recovery plan (NMFS, 2007).

Adult male removal – Objectives and Justification: Aggressive adult male seals, either acting singly or in groups, can severely injure or kill other monk seals of any age or sex, but typically their victims are either weaned pups or adult females. In some cases, researchers have been able to stop the attacks and treat injured seals, but the adult male continues the aberrant behavior.

To enhance the survival of victims of male aggression, it is proposed that, when such males are identified as confirmed or highly suspect aggressors, they may be translocated to alternate sites where they would be less likely to cause harm. Other tools for mitigating male aggression include removal to permanent captivity or, as a last resort, lethal removal via humane euthanasia. Euthanasia would be considered in cases where the identity of male aggressors is certain, or circumstantial evidence overwhelmingly indicates direct participation in such attacks.

Chemical alteration to reduce aggression will be explored in captive monk seals (see Behavior Modification, below) to determine feasibility, safety, and efficacy. If effective, this could be an effective tool for enhancing survival of conspecific seals attacked and killed or seriously injured by aggressive males.

Removal of aggressive males has proven to be a very effective tool to reduce mortality. Johanos *et al.* (2010) demonstrated how removal of known aggressors and "profiled" suspect aggressors rebalanced a male-biased sex ratio and greatly reduced mortality due to multiple-male aggression at Laysan Island. Baker *et al.* (2011) showed that the selective removal of just two seals at French Frigate Shoals (known to be single male aggressors killing pups) greatly reduced losses of pups to this cause of mortality.

Male aggression-related mortality, whether it involves individual or multiple males acting simultaneously, is episodic and unpredictable. Some background low incidence of male aggression causing wounds or death is probably impossible to entirely eradicate. However, the population-level effects, especially when the victims are adult females, are potentially severe. Since 2008, there has been a slight uptick in wounding and losses of females due to multiple male aggression at Laysan Island, and observations of this phenomenon were documented at Nihoa Island in 2010. Single male aggression has caused some wounding and suspected losses of pups at several NWHI sites in recent years. It is therefore very important that the HMSRP be prepared to respond with male removals when the aggressors have been identified or are highly suspected.

The proposed action has been determined to be a Priority 1 intervention action by the Hawaiian Monk Seal Recovery Team (NMFS, 2007). Specifically, action 7.1.2. states "Remove aggressive males, translocate if possible, or euthanize; periodically review criteria for removing aggressive males."

The proposed enhancement activity will result in increased survival of seals, furthering chances for monk seal recovery. Use of an alternative species is not appropriate. The benefits of improving female seal survival far outweigh the individual and population costs of male removals.

Adult male hazing – Objectives and justification

In cases where adult male seals are attacking a conspecific in a way that appears to immediately threaten the victim with death (i.e., by drowning) or serious injury, field researchers may intervene and haze the aggressor away from his victim. This action would be taken conservatively, again only in cases where serious injury or mortality appears imminent.

Males may also be hazed when they are known to be habitual aggressors that have caused harm to conspecifics. In such cases, the male may be hazed away from potential victims (typically weaned pups) even before serious injury or mortality are imminent. This issue arose at Kure Atoll in 2011 and the following description illustrates the need and value of this activity.

At least 10 of 12 weaned pups and three juveniles were attacked by adult male seals in 2011 at Kure Atoll. Two adult male seals (KE18 and KO42) in particular displayed extraordinary aggression towards pups and juveniles. These two male seals individually attacked multiple weaned and juvenile seals repeatedly and ferociously, causing serious injury (wounding include dorsal lacerations, scratches, and puncture wounds, some resulting in inflammation and abscess) and death (two injured pups with severe wounds were not resighted after an initial period immediately following their attacks). KE18 and KO42 males were observed to harass, bite, mount, scratch, chase or hold seven of the pups and the three juveniles underwater; another three pups had injuries consistent with male attack that were not directly observed.

KE18, a 9 year old male, was observed being aggressive toward 9 individuals on 7 occasions between 24 May 24 to 28 July 2011. KO42, a 5-year old male, was observed being aggressive toward 4 individuals on 5 occasions between 31 May 2011 and 19 June 2011.

KE18 met the criteria for removal (lethal or to captivity) above, but this could not be affected during the field season of 2011. In a total of 7 and 4 instances, respectively, NMFS staff hazed KE18 and KO42 away from pups they were attacking or approaching. NMFS thereby reduced the immediate threat posed by the males to the survival of vulnerable pups.

Disentanglement/Dehooking – Objectives and Justification: The objective of this proposed enhancement activity is to alleviate the effects of seal interactions with marine debris or fishing gear, by removing entangling debris or fishhooks which may afflict individual seals, thereby increasing chances of an individual's survival. The removal of entanglement hazards, marine debris and derelict fishing gear from the NWHI has been a major management objective of the HMSRP. In addition, the reduction or elimination of human impacts to monk seals has been a significant management objective of HMSRP in the NWHI. From 1982 through 2010, personnel have actively disentangled seals 209 times. Key publications related to entanglement include Henderson (2001) and Donohue and Foley (2007).

Disentanglement and dehooking have been identified in the Hawaiian Monk Seal Recovery Plan (NMFS, 2007) as Priority 1 intervention actions.

Specific actions are: 2.1 "Continue programs that facilitate the disentanglement of animals," and 6.1.5 "Mitigate mortality by removing hooks from seals."

Supplemental feeding following captive care – Objectives and Justification: Temporary captive care of Hawaiian monk seals in need of medical attention (for example, stranded, injured, prematurely weaned or emaciated seals), can be conducted under the authority of the national Marine Mammal Health and Stranding Response Program (MMHSRP) permit. Thus, captive care of seals in need of medical attention is not an activity proposed in this permit application. However, it is proposed to *complement* captive care with supplemental feeding of seals after they have been released in the NWHI. The concept is to provide a more gradual transition from captivity (where seals will have been fed) to independence (where seals will need to forage for themselves). This may improve the survival prospects of seals following captive care. Such supplemental feeding of wild seals would occur *only* in the NWHI where human presence is minimal. It would *not* be conducted in the MHI, to avoid the problem of these seals approaching members of the public as a food source.

While supplemental feeding *per se* was not recommended in the Recovery Plan, a Priority 1 recommendation is "Rehabilitate malnourished juvenile seals when and where food limitation is apparent to salvage their reproductive potential." To the extent that supplemental feeding will increase the successful reintroduction of rehabilitated seals cared for in temporary captivity, it is a natural extension of this recommendation.

Behavioral Modification – Objective and Justification:
Human- seal interactions and mitigation through aversive conditioning
Hawaiian monk seals have been observed to socialize with humans and their pet dogs on land and in the water (Jenkinson 2011, NMFS unpublished data). These interactions pose risks to seal or human safety when either party becomes aggressive, when people provision seals with food, or when seals take catch from spearfishers or other fishing gear. Human-seal interactions range

from seemingly benign encounters with no physical contact up to violent interactions resulting in injury to people or seals. Repeated interactions may result in seals habitually seeking human interaction, eventually escalating to a dangerous situation. When people intentionally or unintentionally provision seals with food, this may result in particularly strong and rapid habituation. Food provisioning by humans or seals stealing bait or catch from spearfishers or their gear was evident in the histories of 11 of 23 seals that had documented interactions with humans. Sixty-eight unique seals have been documented as hooked or entangled by fishing gear in the MHI during 1988-2011. Because the MHI seal population is apparently increasing (Baker et al. 2011), undesirable human-seal interactions are sure to continue and may become more common. Thus, there is a need to develop and apply methods for mitigating human-seal interactions.

There is little known about shaping monk seal behavior. A few cases where monk seals were hazed have been described, and literature about behavioral modification of other pinnipeds may be relevant to monk seals (Jenkinson 2011). Visual (waving palm fronds) and acoustic (e.g., clapping, yelling) deterrents were applied to a juvenile male monk seal that had been socializing with humans and dogs. However, no behavior change was apparent in this seal after 18 attempts with these techniques over 3 months (Jenkinson 2011). Also, monk seals that have hauled out in undesirable areas such as roadways, paths and tennis courts have been coaxed away with crowding boards and the like. Such hazing was considered successful because the seals were not observed to return to these areas thereafter (NMFS unpublished data).

California sea lions (*Zalophus californianus*) in Washington feeding on salmon at fish ladders were hazed with underwater firecrackers, chased by boat (Gearin, pers. comm.), and acoustic harassment devices (AHD) that produced sounds in the 12–17 kHz range (Greenlaw 1987). Also, taste-aversion conditioning was attempted using lithium chloride. These measures were initially successful in changing the sea lions' foraging patterns but the success was not long-lasting and habituation to the deterrent devices was apparent as predation rates of salmon later increased (Gearin et al. 1988). AHDs, underwater firecrackers, and vessel chase were tested on California sea lions, Steller sea lions (*Eumetopias jubatus*) and Pacific harbor seals *(Phoca vitulina richardsi*) feeding on endangered salmon at Ballard Locks, Seattle, Washington, resulting in apparently rapid habituation (Brown et al. 2007). Cracker shells, rockets, and rubber buckshot were also used on the sea lions but only brief deterrence occurred, followed by apparent habituation (Fraker & Mate 1999; Brown et al. 2007). A pulsed low voltage direct current (DC) (pulse frequency of 2.0 Hz and pulse width at 1000µs) effectively repelled harbor seals 2-3m from a gill net and effectively prevented depredation at a gill-net test fishery on the Frasier River, British Columbia, Canada over a 22-day period (Forrest et al. 2009).

The ultimate goal of the activities proposed is enhancement, yet research in an adaptive management context is needed to identify the most effective tools. Proposed activities involve efficacy trials of aversive tools for understanding and mitigating human-seal interactions. Aversive tools will be tested on seals observed to be interacting with humans in ways that are potentially or actually harmful. Harmful seal behaviors include biting, grasping, mounting and holding a human underwater. Potentially harmful seal behaviors are less defined but include such "gateway" behaviors such as contacting humans, frequently approaching and following them or stealing their catch or bait. These behaviors are believed to demonstrate that the seal is not wary

of human interaction, may be actively seeking human attention or food, and run the risk of eventually becoming actually harmful.

Seals displaying such behaviors will be exposed to unpleasant (but not harmful) stimuli such that they develop a healthy avoidance of close contact with humans. In addition to aversive stimuli, positive reinforcers may be researched and developed to replace the reinforcement of interacting with humans. The efficacy of tools and techniques will be examined in an experimental fashion, and if demonstrated safe and effective, applied as appropriate.

Male aggression and mitigation with GnRH agonists

Behavioral modification techniques may also be useful for mitigating and preventing male aggression-related injury and mortality of juvenile and adult female monk seals. As described above, the HMSRP is requesting to mitigate adult male seal aggression by a variety of means that have been used in the past. Males identified as aggressors may be translocated, brought into permanent captivity or as a last resort, lethally removed. Each of these methods has drawbacks. It would be desirable to develop another tool for mitigating male aggression that was effective, humane, feasible, affordable and reversible.

Translocation works best if the aggressors can be taken somewhere where they do not persist in harming other seals or elicit other problems. In the past, male monk seals were translocated from the NWHI to Johnston Atoll (1984 and 1998) or to the MHI (1994), sites chosen because they harbored few or no other seals. Currently, Johnston Atoll is the only site within the species natural range which has few or no seals. However, past experience suggests that seals taken to Johnston Atoll do not persist there. Permanent captivity is effective, however captive facilities that are willing and able to indefinitely care for adult male monk seals are rare. Lethal removal is also effective, but the HMSRP has used this extreme measure very judiciously and considers it a regrettable last resort. All the above approaches can also be logistically complex and quite expensive, factors which also limit their viability. Finally, in cases where the identity of male aggressors is suspected, but not unequivocal, permanent removal efforts (captivity and euthanasia) are not appropriate.

In the 1990's, some experimentation to chemically alter testosterone levels of adult male Hawaiian monk seals using a gonadotropin-releasing hormone (GnRH) agonist (decapeptyl), was done with both captive and wild seals (Atkinson et al. 1992, 1993, 1998). The results indicated that treated males usually responded by exhibiting lower testosterone levels. However, the studies did not address whether aggressive behavior was reduced. Other drugs (for example, Desolorelin) have also been used in a variety of species to reduce testosterone production and aggression. Research is proposed to better elucidate the potential use of GnRH agonists as a tool for mitigating adult male monk seal aggression. Research would involve trials with captive monk seals. If the method proves effective, it could be used as an enhancement alternative to temporarily alter aggressive behavior of specific male seals.

Vaccination – Objectives and Justification: Current information suggests infectious disease is not limiting recovery of the Hawaiian monk seal. However, the species is rare, has very low genetic diversity and may have been buffered from exposure to many mammalian diseases due to

its isolation in the Hawaiian Archipelago for millions of years. Together, these factors raise great concern that outbreaks of diseases to which monk seals have not been previously exposed could have devastating impacts.

Proactive efforts to mitigate the potential or eventual negative effects of infectious disease on monk seals include vaccination studies to determine the safety and efficacy of vaccines against specific pathogens considered most likely to spread to monk seals (e.g., Morbillivirus and West Nile Virus). Captive studies would include both monk seals and surrogate species, and potentially free-ranging Hawaiian monk seals. If such research indicates that the vaccines are safe and effective, they may be administered preventatively or in response to an outbreak, to wild or rehabilitating seals.

Epidemic diseases (referred to as epizootics when occurring in animals rather than humans) are diseases that occur at a time or place that they do not usually occur, or with a greater frequency than expected in a certain period. Severe epidemics may reduce host population density to such an extent that stochastic events or previously unimportant ecological factors may further reduce the host population size (Harwood and Hall 1990). For example, canine distemper dramatically reduced black-footed ferret (*Mustela nigripes*) populations in Wyoming, bringing them to extinction in the wild (Thorne and Williams 1988); and, avian malaria reduced native Hawaiian honeycreeper (*Hemignathus parvus*) populations to such small numbers that many were finally eliminated by predation or habitat loss (Warner 1968).

Infectious diseases, especially those that are newly introduced to naïve populations of animals, can cause mass illness and mortality. The best means of preventing the spread of infectious disease among animals are vaccinations. Vaccines are available for two viruses that have been identified as high risks to Hawaiian monk seals: morbillivirus and West Nile virus. Background surveys conducted on Hawaiian monk seals support that they remain naïve to both viruses. These two viruses are the current focus of vaccination research and response planning for Hawaiian monk seals.

Morbilliviruses—These viruses, specifically phocine distemper virus (PDV) and canine distemper virus (CDV), have caused mass die offs of phocids. During 1988, approximately 18,000 (70% of the population) harbor seals (*Phoca vitulina*) in Europe died from PDV infection (Heide-Jørgensen *et al*. 1992). A second outbreak of PDV occurred in the North Sea in 2002, which killed over 20,000 harbor seals (Jensen *et al*. 2002). Outbreaks of canine distemper (CDV) killed 5-10,000 Baikal seals (*Pusa sibirica*) in 1987-1988 (Grachev *et al*. 1989), 10,000 Caspian seals *(P. caspica)* in 2000 (Kennedy *et al*. 2000) and may have been responsible for the deaths of 2,500 crabeater seals (*Lobodon carcinophagus*) in the Antarctic in 1955 (Laws and Taylor 1957). While a morbillivirus was isolated from Mediterranean monk seals (*Monachus monachus*) that died during an epidemic, its importance relative to biotoxins in causing mortality remains controversial (Hernandez *et al.* 1998). While the susceptibility of Hawaiian monk seals to morbilliviruses is unknown, due to the devastating effects these viruses can have on phocids, there is a need to better understand and prepare for such an event in Hawaii.

West Nile Virus—This virus caused the death of a captive monk seal at SeaWorld San Antonio, Texas, and has caused mortality in captive harbor seals in the mainland U.S. To date this virus

has not been identified in wild marine mammals, although it is present along the eastern seaboard and southern California. This mosquito-borne virus is currently not present within Hawaii, and the State has rigorous surveillance and response plans for this virus due to its public health importance. Although neither single cases of disease nor epidemics of West Nile Virus have been reported in wild marine mammals to date, the death of a monk seal in Texas from this infection indicates monk seals are susceptible. Thus, the possibility of extensive mortality in monk seals exists if the virus were to be introduced to Hawaii, warranting a response plan to such a scenario.

Available vaccines—Vaccines currently used for prevention of viral diseases in domestic animals can be divided into three types:

- Vaccines based on a dead inactivated virus;
- Vaccines using live attenuated viruses; and
- Vaccines consisting of recombinant viruses.

Vaccines using a dead virus are considered the safest because the virus cannot replicate in the host or cause disease; however, this lack of replication often means that the immune response generated following vaccination is short-lived and may not be protective. Live vaccines typically generate the most effective immune response. When used in species other than the one for which the vaccine was developed, live vaccines present the risk of the virus replicating in the host and either causing disease in the vaccinated animal, or being shed in secretions and becoming infective to contact animals. One vaccine proposed for use under this permit is an inactivated West Nile virus vaccine (Innovator, Fort Dodge) that has been used regularly to date on Hawaiian monk seals in captivity in San Antonio, Texas, with no adverse reactions observed (Workshop to Evaluate the Potential for Use of Morbillivirus Vaccination in Hawaiian Monk Seals, Final Report 2005).

Recombinant virus vaccines use a vector virus that does not typically infect the target host but expresses antigens from the pathogen of interest to stimulate an immune response against it. A recombinant vaccine to CDV (monovalent recombinant canary pox vector expressing canine distemper virus antigens, Purevax, Merial) licensed for use in ferrets in the U.S., is now used extensively in zoological collections (Bronson et al. 2007) and is proposed for use in research and enhancement activities under this permit. It is the only distemper vaccine recommended by the American Association of Zoological Veterinarians for use in non-domestic carnivores including mustelids (http://www.aazv.org). It is approved generically for animal use in the State of Hawaii. Safety and efficacy trials with this CDV vaccine have been conducted on four captive harbor seals and on one captive Hawaiian monk seal. These preliminary studies demonstrated that the vaccine is safe, and antibodies to canary pox were detected after a second (booster) dose. This vaccine has also proven to be a safe and effective prophylactic treatment for captive southern sea otters (*Enhydra lutra nereis*) (Jessup et al. 2009).

Use of dead seal flesh for bait for predation mitigation at French Frigate Shoals – Objectives and Justification—In an effort to mitigate predation of Hawaiian monk seal pups at French Frigate Shoals, HMSRP may attempt to remove Galapagos sharks at select islets in the atoll during the breeding season (April to September) under separate permits issued by the

Monument. Predation mitigation is recommended in the species' Recovery Plan and is deemed necessary for the recovery of the French Frigate Shoals monk seal subpopulation (NMFS 2007). Each year for the last decade, 15-28% of the incoming monk seal cohort is lost to shark predation at this important subpopulation (NMFS 2010).

To capture large adult Galapagos sharks, we will bait hooks primarily with large tuna heads and shark remains. However, on the rare occasion that monk seal flesh from dead animals (after necropsy procedures and sampling) is available, we seek permission to be able to use this tissue as bait. Relatively large pieces of tissue of skin/blubber/muscle from dead monk seal pups or other deceased older individuals would be used. Typically, when dead monk seals are found on the beach at FFS, HMSRP conducts a necropsy, samples tissues, takes photographs and then buries the remains. By using such tissue for bait rather than burying it, our intent is to help protect remaining live pups from shark predation.

NMFS unpublished data and NMFS field reports describe Galapagos sharks taking fresh dead seal tissue or placenta while staff fished with hand lines from Trig Island in years past. Galapagos shark took monk seal bait on one occasion each in 2001 (July 5, 2001), 2002 (June 23, 2002), 2003 (August 3, 2003) and 2005 and these sharks were then culled. On an additional 12 occasions, between 2003-2006, monk seal tissue was used as bait but did not result in catching Galapagos sharks.

Based on experiences from 2001-2006, the catch per unit effort for using handlines with seal flesh is higher than using handlines with fish bait. Seal flesh resulted in 3 catches out of a total 15 attempts, which translates into a 20% success rate, better than what has occurred with fish bait during these years when handlines were used. This suggests that using seal flesh as bait holds promise.

We expect that the carcasses of up to 8 pups and 1 adult (either sex) may be made available to us during the course of the breeding season at French Frigate Shoals annually. We do not anticipate any impacts on the greater monk seal population with the exception of the benefit of being better able to capture sharks near islets with pups. This should lead to increased protection and survivorship of the pup cohort. Use of seal flesh as bait in the past did not result in increased shark activity or predation incidents on pups.

It has been hypothesized, but not confirmed, that the presence in the waters surrounding Trig Island (FFS) of dead pups drowned by aggressive males may have attracted Galapagos sharks in the late 1990's and contributed to these sharks focusing on monk seal pups as prey. Whether or not this indeed contributed to the increased predation observed after the late 1990's, the presence of free floating dead whole weaned pups bears little relation to the proposed use of dead seal flesh as bait. Most notably, the seal flesh would not be used as chum released into the water. Rather, it would be attached to a hook and line, already proven sufficient to capture Galapagos sharks. Thus, any Galapagos shark that ingested the bait would be killed and pose no further threat to monk seal pups.

Project Description/Methods

A table delineating proposed research and enhancement activities (i.e., takes), including the number of seals authorized to be affected or potentially disturbed by an activity at a specific location, is in Appendix B (take tables). While the activities are separated in the table, it is possible that individual seals could be taken by more than one of the activities. Because the activities would occur at different times, both within a given year as well as over the 5-year duration of a permit, it is not possible to predict the degree of 'overlap' of activities which may impact a given animal, or account for such multiple takes when providing annual permit summaries. The numbers of animals that may be affected are listed under separate activities, and the likelihood of multiple activities occurring on the same animal annually is identified in the take table of Appendix B. A separate take table (Table 2) for activities proposed with captive seals is included in Appendix B. Captive monk seals could be used to test or validate any of the methods described below.

As refinements in manufacturing of research technologies progress, new diagnostic tests are developed, and improvements in research methods are made, we propose to incorporate such improvements into our field research as long as the impacts to the seals remain the same or are reduced. For example, new telemetry tag types may be used for the same objectives as long as the devices have the same or lesser footprint on the seal. The use of new and improved technologies would be applicable to any of the methods described below. We will notify the NMFS Permits Division of any such introduction of new technologies.

Population Assessment/Monitoring: Virtually all NWHI monk seal population assessment data are collected during annual field seasons typically occurring within March to September. Reports of activities are published annually (e.g., Johanos and Baker 2007). Data from seals in the MHI is collected year-round by HMSRP staff, assisted by volunteer reporting (including a call-in number for public sightings and dedicated volunteer efforts).

Population Monitoring Methods: Seals could be taken annually by disturbance from all monitoring activities (air, vessel, ground), at any haulout site within the Hawaiian Archipelago or Johnston Atoll. The annual maximum take totals (see take table for site-specific numbers) are based upon levels of disturbance takes in recent years at each location, adjusted to account for (1) realized or anticipated potential increases in some locations (e.g., MHI) or likely continued decreases in abundance, and (2) additional monitoring required to support new and expanded research and enhancement activities above and beyond recent levels. Seals of any age and either sex may be taken by disturbance, including pregnant females and nursing pups. Disturbances could occur during ground observations of seals (e.g., censuses) by researchers or unmanned vehicles, aerial and boat surveys including unmanned aerial and amphibious vehicles), as well as from installation and repair of remote video cameras.

The monitoring technique is almost exclusively personal human observation, either on shore or via aerial or vessel survey. Most observations are made by biologists walking on shore adjacent to seals. Observations are made using binoculars. Photographs of identifying scars or other noteworthy conditions, such as injuries, are documented with digital cameras equipped with telephoto lenses. Data recorded on land-based surveys include date, time, location, and a variety

of information about each individual seal encountered (size, sex, tag information, bleach marks, body condition, molt status, association with other seals, injuries, behavior (e.g., for monitoring aggressive adult males), and reactions of seals to researchers.

Aerial surveys involve censusing Hawaiian monk seals from either a helicopter (MHI and Kaula Rock) or a fixed wing aircraft (Necker and Nihoa Islands), or unmanned aerial vehicles. Minimum distance from the manned survey aircraft to seals would be 500 feet and lower (e.g., to 300 feet) if necessary (vector combination of vertical and horizontal distance) for fixed winged aircraft and 250 feet for helicopters. Typically, surveys are conducted by flying offshore of shorelines until a seal is spotted, then circling (or hovering) to observe and photograph. Past observation demonstrates that Hawaiian monk seals rarely respond or appear to notice aircraft, perhaps due to the lack of aerial predators in their evolutionary history.

To further enhance population assessment and monitoring, the HMSRP proposes to use unmanned terrestrial or amphibious vehicles, aerial vehicles and video cameras (described below) to monitor several sites that are difficult or impossible to access, such as Necker, Nihoa, and Niihau. HMSRP plans to test the use of quadrocopters, which are lifted and propelled by four rotors controlled by varying the relative speed of each rotor to change the thrust and torque. These vehicles use an electronic control system and electronic sensors to stabilize the aircraft; and, because of their small size and agile maneuverability, they can be flown indoors and outdoors. These could be flown as close as 10 ft from seals.

The advantages of stability with the current generation of small-scale quadrocopters are the vehicles are safer to interact with in close proximity to objects. By enclosing the rotors within a frame, the rotors can be protected during collisions, permitting flights indoors and in obstacle-dense environments, with low risk of damaging the vehicle, its operators, or its surroundings. This includes low risk to monk seals and birds should the helicopter crash or be struck in flight.

The quadrocopter would be remotely controlled continuously (not programmed for a mission) and would stream live video during the operation from 1 – 2 cameras mounted on the aircraft. This allows operators to control the craft as well as take digital stills or video of monk seals. There are a variety of quadrocopter systems that are commercially available and most are similar in size and noise production. More advanced models offer greater stability, higher resolution cameras, greater range, or longer battery life. The specifications of the system the HMSRP will be trying first are below. Additional unmanned aerial vehicles may be employed over the 5-year duration of the permit as technology improves. Such remote controlled vehicles would be of similar specifications or smaller/quieter and would be used for the same purposes.

Running Speed: 18km/h

Weight
- without the hull: 360g
- with the hull: 400g

Dimensions
- without the hull: 45 x 29 cm
- with the hull: 52,5 x 51,5 cm

Battery
- Lithium polymer battery (3 cells, 11.1V, 1000 mAh)
- Charging time: 1h30
- Running time: 15 mn

Embedded computer system
- Processor ARM9 RISC 32 BITS - 468MHz
- DDR RAM 128 Mo - 200 MHz
- Wi-Fi b/g
- Linux OS

Ultrasound altimeter
- Emission frequency: 40kHz
- Range: 6 metres

Front Camera
- 93° wide-angle diagonal lens camera, CMOS sensor
- Video frequency: 15fps
- Resolution: 640x480 pixels (VGA)

Vertical Camera
- 64° wide-angle diagonal lens camera, CMOS sensor
- Video frequency: 60fps
- Resolution: 176x144 pixels (QCIF)

Autonomous, amphibious rover vehicles could be used improve assessment and monitoring operations in certain situations (crowded narrow pupping beaches, islands that are difficult to land on). Terrestrial rovers with mounted video cameras could be used to identify and photograph monk seals or potentially assess injuries. The use of rovers has not been tried with monk seals so their application would be in two phases. The first would be to test the vehicles suitability in near shore environments for collecting current or better quality assessment data and monitor their potential for disturbing monk seals and other wildlife. If the rovers prove successful they will be utilized more broadly on an “as needed” basis. Most data collection would still occur using traditional methods.

The typical rover will be a track-type vehicle equipped with molded polyurethane tracks although other versions can be equipped with foam-filled tread tires and/or wheel-leg (Wheg TM), type propulsion. The vehicles could have a still camera mounted on a section of PVC housing with Sensor Processors (SP) in aluminum pressure housing, and antennae for GPS navigation. Vehicle construction would use non-magnetic materials as much as possible. Chassis would typically be constructed of heavy gauge laser-cut aircraft grade aluminum with welded aluminum seams. Wheel type propulsion would typically use four IG32P 265 RPM 24DC gear motors (one per wheel), or similar. Power would be provided by a 22.2VDC 3000mAhr Li-ion drive battery with a run time of 2-8 hours depending on use. Fuse blocks, terminal blocks, charging ports, switch, or other options (e.g., camouflage, GPS navigation, solar panels, etc.), can all be integrated into the design. Camera may use an 11.1VDC 5000mAhr Li-ion battery to power a color video camera with integrated low profile pan and tilt system and 10 LED IR night

vision ability. Data would be sent wirelessly from the vehicle to a computer enhanced with a frame grabber to store video sequences and photographs. Vehicle length would average between 46-63 cm, width 38-55 cm, and a weight range of 12-20 kilograms (within average person's ability to carry), yet heavy enough to withstand most marine animals. Total height would typically be 25-31 cm with chassis ground clearance about 3-4 cm. Vehicle could operate on land or water at a nominal speed range of 0.5-0.6 meters per second with a turn rate of 60-65 degrees per second. Faster speeds are possible but are at the expense of battery duration and diminishing stability. A covering that resembles a rock or turtle shell may be used to reduce the likelihood of a seal being disturbed by the vehicle. The minimum distance rovers may approach seals is 1 m.

Boat surveys typically occur in areas where biologists cannot access land or where sand spits occur that are too small to access. In the NWHI, the platform is an 18 foot whaler or similar small boat that circles a haulout site from a minimum approach distance of approximately 10 meters. In the MHI, the boat platform varies opportunistically, depending upon what agencies or charter vessels may be traveling to inaccessible areas where observations are important (e.g., remote areas of Kauai, Kahoolawe). The minimum approach distance is the same as in the NWHI, 10 meters.

In some cases, the HMSRP proposes to use video camera systems, such as in remote locations. For example, three remote video cameras will be potentially installed at Nihoa Island to monitor resident Hawaiian monk seals; and such cameras may be deployed at additional locations in the Hawaiian Archipelago (e.g., to increase monitoring at other remote locations). The camera(s) will be placed on the cliff faces or edges along the sand beach that supports the majority of an island's seal population and pupping. Video cameras are solar and battery-powered, 7" x 11 "x 4" in size, and a dish is installed for transmitting the data. At least one camera will have pan-tilt-zoom functionality; individual seals can then be better identified from their identification tags and markings. A weatherproof box containing a server and transmission relay station will be set up near the power system to allow remote control of camera and recording systems via the Internet. Video signals are wirelessly sent to a receiver at the site and transmitted via satellite to Honolulu, where they can be accessed via the Internet. The installation work will typically occur over two days with field biologists present, and regularly scheduled maintenance of the camera systems will be attempted annually during field camp deployment cruises (approximately 1-2 times/year). Additional repair of the systems may be necessary in the event of a malfunction. In the case of repair, a monk seal biologist will again accompany engineers to ensure minimal disturbance; the timing of a repair trip will depend on contract vessel or NOAA ship availability.

Population Monitoring Mitigation: Observers remain as far away as possible from seals during monitoring activities to obtain the necessary data, using binoculars and telephoto lenses as necessary for documentation. All field staff are trained to be unobtrusive and to remain low to the ground whenever seals may alert to human presence. Seals are specifically given a wide berth when they are especially susceptible to disturbance, such as lactating females or molting individuals. For every observation collected by the HMSRP, a note is coded if a disturbance to the animal occurred, but the individual disturbed is not necessarily identified. A seal is considered disturbed if it moves in response to having seen the observer. The level of disturbance is assigned based upon how far the animal moved, or if it fled into the water.

Boats transiting lagoons would not divert from straight-line paths necessary to transit between the islands, and boats would avoid landing on beach areas where seals are in the immediate vicinity. Monk seals rarely react to aircraft but in the rare occurrences when a seal may appear to respond to aircraft presence, the altitude is increased until the seal settles down. It is unlikely that monk seals would be disturbed by quadrocopter presence or noise unless the craft was flown too close. Surveys could be successfully completed without approaching closer than 10 feet. The HMSRP will closely monitor seal reactions to the quadrocopter in the first 5 trials of the technology; and, if disturbance is apparent via behavioral displays (e.g. vocalizations, fleeing into water), researchers will adjust the minimum approach distance to be greater than the proposed 10 feet.

For video installments and maintenance/repair, at least one monk seal biologist will accompany the engineers to monitor seal response to the installation at all times. Researchers will minimize disturbance to seals by skirting the edge of the beach during the installation procedure. USFWS will be consulted on appropriate mitigation to minimize disturbance to any protected bird species and the HMSRP will endeavor to have one USFWS biologist present during the installation as well. Though the installation of the camera system at Nihoa may allow HMSRP to decrease the number of surveys for population assessment for years to come, there is some chance that monk seals will be disturbed while scientists and engineers are accessing the site, installing the system and performing maintenance. Seals present on the beach during the installation will be continuously monitored. In the event that a mother and pup pair is observed to separate due to our activities we will attempt to reunite them.

Flipper and PIT Tagging and Measuring

Tagging and Measuring Methods: A total of 620 Hawaiian monk seals of any size or sex (except lactating females) may be tagged or retagged annually within the Hawaiian Archipelago and Johnston Atoll. Flipper tags are applied to weaned pups of both sexes and to older individuals that may have not been tagged previously. Tags would be re-applied to individual seals whose tags have become lost, broken, or excessively worn, in order to maintain the individual identities of these animals.

In some cases at the end of a field season when not all of the pups have weaned, nursing pups that are handled during interventions to reunite them with the mother or place with a foster mother would be tagged in order to facilitate identification the following year. Similarly, pre-weaned pups may be tagged very rapidly and with minimal restraint using metal or plastic tags with a piercing side that attaches to a second element as described below.

Seals typically would be manually restrained by hand, or in a stretcher net, hoop net, or throw net and tagged with two plastic Temple Tags®. Temple Tags measure 4 cm x 2 cm and are inserted through holes punched in the webbing between two digits of each rear flipper. During retagging old broken or unreadable Temple Tags may be removed. Restraint time would average ~5 minutes and would not exceed 15 minutes.

After flipper tags have been applied, but while the seal is still under restraint, a single PIT tag would be injected. Most PIT tags would be injected just below the blubber in the lumbar area,

approximately 5 inches lateral to the dorsal midline and approximately 5 inches anterior to the base of the tail. However, alternate sites on the seal's posterior may be tested. The injection site would be cleansed with Betadine® and alcohol prior to PIT injection.

Seals would then be measured (length and girth) using a flexible cloth tape. No sedating drugs would be administered to the seals during restraint for flipper tagging/measuring. Flipper plugs, resulting from punching the webbing of hind flippers to apply Temple Tags, would be retained and banked for DNA analysis. Plugs are fixed in DMSO, returned to the laboratory in liquid nitrogen, and banked in ultracold freezers.

Currently, pups are not tagged until they have weaned so as not to disturb mother-pup pairs. However, nursing pups are frequently bleach marked, which can be accomplished with little or no disturbance. These marks are not visible for more than a few weeks unless they are applied near the end of the nursing period when pups have already molted. Here, it is proposed to apply a single tag to some pre-weaned pups. This would only occur in cases where pups have not and will not wean prior to the end of field camps (otherwise, researchers would wait until weaning as usual to apply tags). Tags fitted into purposed-designed pliers that pierce and attach or crimp (e.g., monel steel or plastic tags such as All-Flex, Dalton, or Roto) in a single rapid motion would be used. These tags are likely less durable and readable in the long term than Temple tags, however surviving pups may be recaptured as yearlings (the subsequent year) and retagged with the longer-lasting Temple tags.

It is anticipated that less than 25 pre-weaned pups would be tagged in this way annually. Tags would be applied by sneaking up to a sleeping pre-weaned pup, when it is relatively far from its sleeping mother. A single rear flipper would be rapidly held manually and the tag applied between two digits along the trailing edge of the flipper. Tagging will be completed in less than 10 seconds, typically in 1-2 seconds. The researcher would then immediately vacate the area to minimize disturbance but observe to confirm the mother and pup remain together. Based on observations following enhancement activities (e.g., fostering or reuniting mother-pup pairs), incidental research disturbances in the past, and disturbance by members of the public of mother-pup pairs in the MHI, these brief and covert tagging events will not cause mother-pup separation, premature abandonment or other such deleterious effects.

Finally, tagging seals older than pups may also be accomplished using Roto and monel tags as described above for pre-weaned pups. This method may be selected to facilitate long-term identity of seals when full capture and restraint is not safe or practical.

Tagging and Measuring Mitigation: Because of the critical importance of adult female and pup survival, lactating females would never be tagged during the suckling period. As described above, pre-weaned pups may be tagged if they have been separated from their mother and researchers were handling the pup as an intervention. Some pre-weaned pups may also be rapidly tagged with minimal restraint as described above. This would only occur when 1) the pup will clearly not wean prior to researchers' departure from the field site for the season and 2) the pup and mother can be approached safely and with minimal disturbance (both sleeping and separated some distance). Moreover, tagging molting seals would also be avoided due to the higher physiological demands to animals that are shedding and regrowing their entire epidermis.

The capture team has a briefing prior to an event to discuss roles of each team member and contingencies and responsibilities in the event of unanticipated results or action by the animal. Researchers would minimize stress from captures and restraint for tagging/measuring by keeping the handling procedures as short as possible and cooling the animal with water whenever deemed prudent. For routine tagging which entails short restraint times, administering sedatives presents more of a risk to the seals than the stress that the sedative would relieve, and the procedure would add significantly to the restraint time. Similarly, a local anesthetic such as lidocaine could be administered to relieve the transitory pain experienced by the seal when a tag is applied, but this would add to the restraint time, presenting a higher risk. Prior to any animal capture the site would be evaluated for presence of environmental hazards that could present a risk of injury to the animal or the handlers. For example, seals would not be restrained or tagged if they are in proximity to rock ledges or dangerous substrate.

Seals are observed for a minimum of five and up to 20 or more minutes after being marked, disentangled, or handled for any reason (tagging, sampling, instrumentation involving sedation) to ensure they resume normal behavior (either going into water or resuming normal respiration rates on land). Seals going into the water would be observed until they are out of sight. Regular patrols and censuses of the area would be conducted to resight and monitor tagged individuals.

Procedures requiring physical contact with seals include precautions to ensure that humans handling seals do not inadvertently transfer pathogens between animals. All personnel who come into contact with the seal would wear protective clothing (e.g. coveralls, gloves, booties) which is either sterile or has been washed/disinfected. All instruments/gear would be cleaned by washing thoroughly with soap and water and then disinfecting with 70% Isopropyl Alcohol, or a 1:20 solution of Clorox©, for a minimum of ten minutes. This includes such items as nets, tape-measures, calipers, pit tag applicators, tag hole punchers, cooler, and buckets, or other items touched after the capture and before washing hands. All personnel involved in restraining seals would, prior to handling another animal, wash their hands in anti-bacterial soap or use hand sanitizer, don clean coveralls and a fresh pair of latex gloves (and cotton over-gloves) per preference.

Deploying Sonic-Flipper Tags to Weaned Pups

Sonic Tag Deployment Methods: Small sonic tags (attached to a flipper tag) could be deployed on up to 35 weaned pups at French Frigate Shoals annually. Sonic tags would be deployed concurrent with standard flipper tagging of pups when they wean, including those pups that may be relocated to safer areas to mitigate pup loss due to shark predation. All pups encountered are flipper tagged at weaning, and those that wean in high-risk areas at French Frigate Shoals where sharks predate on pups are captured and transported via small boat directly from weaning site to release site within the atoll (see translocation section below).

The sonic tag would be attached onto one additional flipper tag during standard tagging procedures, and it is estimated to take less than a minute to apply an additional tag. The sonic tags are 24mm long and weigh 3.6 grams in air and 2.2 grams in water. The sonic tag is about the size of the Temple tag and would be attached to the Temple tag with two small zip ties and

epoxy. The transmitters ring at 69 kHz. The sonic tag eventually would break off from the Temple Tag and would not be recovered.

Sonic Tag Deployment Mitigation: Mitigation measures employed during flipper tagging would apply to sonic tagging, as these activities would be conducted concurrently using the same methods to apply tags.

Bleach Marking

Bleach Marking Methods: Up to 1,495 seals in the population may be bleach marked annually, as described by each island/atoll in the Appendix B take table. The technique for marking monk seals in the wild involves sneaking (often crawling) up to a sleeping seal and applying a unique identifier (usually a number) to the seal's pelage on the back or side. A bleach "girdle" is also applied over the seal's circumference in the vicinity of the tail. The purpose of the girdle is to facilitate subsequent detection by observers that a seal has been bleached, even if the animal is lying on the previously applied number.

Bleach to be used would be a commercial cosmetic hair lightener, and would be applied from a squeeze applicator (similar to a condiment dispenser), usually without disturbance to seals asleep on the beach. Marks remain on the seals' pelages until the annual molt, with a maximum duration of one year.

Bleach Marking Mitigation: Bleach is never applied to a part of the pelage that the seal could reach with a fore flipper, to ensure that the animal cannot rub any bleach on its face or in its eyes. Only seals that are asleep would be bleached. Molting seals, which are more restless and subject to disturbance, would be avoided. Seals would not be bleach marked if they are sleeping in close proximity to other sleeping seals or basking green turtles, or if extrinsic factors exist that could threaten their welfare if they are startled (e.g., rocks). Seals swimming in the near shore area sometimes approach another seal, which is in turn being marked. In such instances, the swimming seal may notice the biologist and vocalize, alerting nearby animals. Researchers would minimize effects on non-targeted animals by avoiding marking seals when other seals are in the immediate vicinity, where they might be startled.

Bleach marks are photographed just after application to facilitate re-identification and seals are observed for five minutes after being bleach marked to monitor their behavior and assess the likely efficacy of the mark (bleach is not likely to have a good effect if the animal goes into the water shortly after marking or the animal may roll onto the mark before the bleach takes, rendering the mark illegible). Follow-up sighting records are maintained of marked seals throughout the field season.

Health Assessment/Disease Investigation and Treatment

Health Screening and Treatment Methods: Screening would include collections from (1) animals displaying debilitation, emaciation, or abscesses, which may be symptomatic of a disease or a disease process; and (2) healthy seals. Appendix C lists the drugs the HMSRP proposes to use for sedating seals and responding to adverse reactions from sedation or restraint,

and for treating seals in the wild. This appendix includes the drug name, dosage and route of administration, purpose, effects, and pharmacological information for each drug proposed. Appendix D provides protocols for treating abscesses and administration of antibiotics.

Up to 30 unhealthy/ill and 100 healthy seals of any age/sex may be sampled annually (excluding lactating females with pups and nursing pups). Up to 10 moribund seals may be humanely euthanized or die incidental to capture for sampling activities over the duration of the permit.

The type of samples collected from injured, ill, or otherwise debilitated seals, as well as the decision on site whether to use sedation on the animal, would be at the discretion of the attending veterinarian. Depending upon the condition of the animal, symptoms it is displaying, and an assessment of the animals' tolerance to restraint, samples would include up to 105 ml blood, viral and microbial swabs from all body orifices and wounds, blubber biopsies, ultrasound, and morphometrics (girth and length), and weight. Seals would be flipper/PIT tagged if not previously tagged. Seals may be recaptured for subsequent health sampling and treatment as warranted per veterinary recommendation.

Weaned pups and juvenile seals are sometimes observed in the wild with large dorsal lumps, which are in fact abscesses resulting from infection of scratches or bites inflicted by aggressive adult males. These abscesses sometimes eventually open and drain, but also may remain closed while the condition of the affected animal deteriorates. Treatment may involve manually lancing the abscesses and flushing them with water and hydrogen peroxide or similar disinfectant. Seals are also often observed with wounds inflicted by sharks, which may be treated with topical disinfectants. Long-acting antibiotics (e.g., Ceftiofur) may also be used to treat such infections as prescribed by attending or consulting veterinarian(s) (Appendix D). An unlimited number of seals may be treated (i.e., as warranted for the health of the seal), separate from health screening.

Procedures for sampling of healthy seals differs from those used for sick seals. Sedation, if necessary, would be with intravenous (IV) diazepam or intramuscular (IM) midazolam (see Appendix C for dosages). After the seal has become sedated, up to 105 ml of whole blood would be collected from the extradural vein using a standard syringe and external T-connector. Microbial and/or viral swabs would be collected from the following sites: eyes, nares, mouth, anus, genital orifice, and external wounds. Two blubber core samples (through the full depth of the blubber layer) would be collected from the dorsal pelvic region using a sterile scalpel and 6 mm biopsy punch. Seals would be weighed, morphometrics and ultrasound taken, and flipper/PIT tagged if not done previously. Total handling time would depend upon the procedure, but would range from 5 to 20 minutes.

Blubber depth measurements using a SonoSite portable imaging ultrasound (SonoSite, Bothell, WA) would be collected by applying light pressure to the skin to obtain images along the lateral side and dorsum of the animal. Blubber depth measurements of seals may occur during health assessments, instrumentation, flipper tagging, and de-worming. Measuring blubber depth provides information on body condition and reflects changes in fat deposition and growth via lean mass.

Milk may be collected opportunistically from lactating females during health assessments, only in rare occurrences if the female has lost or abandoned her pup (and the pup could not be reunited). Milk would be collected by manual expression of the teat, but no hormones (e.g. oxytocin) would be administered to facilitate milk let-down. Milk would be collected in plastic containers and frozen at -20˚ F. Analyses would include, but not be limited to, proximate analysis of fat, protein, water, and ash.

A total of two (2) vibrissae for stable isotope and hormone analysis may be collected annually from all animals captured for flipper tagging, instrumentation, biological sampling or some other intervention (e.g., dehooking, treatment of abscesses). The method of collection will vary depending on if the animal is sedated or not. Pulling, rather than clipping, vibrissae is preferable because clipping results in an unknown length remaining attached to the seal and obtaining the root of the vibrissae, representing the most recent growth, for analysis is preferable. Vibrissae will be removed by pulling only from sedated animals and will be clipped at the base with small scissors if the animal is being restrained without sedation. Vibrissae will be pulled by gripping with forceps or fingers and pulling forcefully in one smooth motion. Seals would only have up to two vibrissae sampled once annually.

Appendix E provides detailed protocols for sample collection, processing, and analysis. All samples would be labeled with the identity of the animal being sampled, as well with standard collection data. Swab samples would be preserved via standard methods appropriate to the microorganism being cultured. Whole blood would be spun in the field and serum would be frozen in liquid nitrogen. Blubber biopsies would also be frozen in liquid nitrogen. Samples would be submitted to contractors for the following analyses, including but not limited to: tests for brucellosis, toxoplasmosis, leptospirosis, canine distemper virus, phocine distemper virus, herpesvirus, morbillivirus, environmental contaminants including heavy metals and hyrdocarbons, and fatty acid composition. Any samples not destroyed during analyses would be retained in ultra-cold storage by the HMSRP or a permitted partner (e.g., NIST). For export, appropriate CITES permits would be acquired.

Lethal Take of Moribund Animals
Up to 10 moribund/unhealthy seals of any age/sex may be humanely euthanized or die incidental to handling over the 5-year duration of the permit. Euthanasia may occur if an experienced on-site veterinarian determines that there is a high probability of the death of the animal due to the injury or disease condition. In such instances, seals would be captured, sedated, and biologically sampled as described above for health assessments. Thereafter, seals would be injected with a lethal dose of Beuthanasia® (sodium pentobarbital) into the extradural vein at a dose of 1 ml/10 lb. Immediately after the animal has succumbed, a complete necropsy would be conducted, with samples saved from all major organs. Because of the presence of barbiturates in the carcasses, all soft parts not retained would be collected in plastic bags for subsequent environmentally safe disposal (e.g., incineration).

Foraging Investigation / Instrumentation

Instrumentation Methods: HMSRP investigates the foraging ecology of the Hawaiian monk seal using instruments attached to the seals dorsal pelage to determine location, diving parameters

such as depth and duration, characteristics of foraging substrate, and in certain instances actual prey identification and foraging behaviors. Telemetry instruments may be applied annually to 60 of the 100 healthy animals sampled for health assessments as described above. Instrumented seals may be may be taken twice to remove the instrument and re-sample and/or if they display symptoms of illness after the first sampling.

A variety of instruments used are specified in Table 4 below, ranging in total combined weight from 27 g to 1 kg (air weight) and would depend on the size of the seal to be instrumented and past deployments known not to have a deleterious effects on seals (see Baker and Johanos 2002; Littnan et al. 2004). Location would be anywhere in the Hawaiian Archipelago.

Seals are captured and restrained using a hoop net or a stretcher net. Sedation with diazepam or midazolam, and administration of atropine, would be based upon the discretion and clinical judgment of the on-site veterinarian. Biological samples would then be collected, as described in health/disease screening above.

Once samples have been collected, an instrument package would be glued to the dorsal pelage using epoxy adhesive. Pelage at the attachment site may be pre-cleaned with a rag wetted with acetone depending on the condition of the seal's fur. Instruments would be attached to the dorsal fur near the shoulder of the seal with a low exothermic adhesive (e.g., Devcon 10 Minute Epoxy Clear). Instruments are either recovered or fall off before or during molt. Seals would be monitored after release for normal behavior. HMSRP's experience with sedated seals is that the animals recover from sedation 15-20 minutes after administration of the diazepam. If deemed necessary by the attending veterinarian, before the procedures are completed additional sedatives may be administered. Total restraint time would average approximately 25 minutes, and would not exceed 60 minutes.

Table 4. Types of instruments to be applied to Hawaiian monk seals. Additional instrumentation may be added as new technologies and tags become available. Such instruments would fall within the specifications below and would be used to enhance data collection for the purposes/objectives in this application. Use of new tags not described here will be provided to the Office of Protected Resources.

Instrument Type	Specifications	Application	Approximate Duration of Deployments	# Deployed/Year
MK9 TDR	6.7 cm x 1.7 x 1.7, 30 g in air	Recording dive behavior	Up to 10 months	Up to 20
MK10 GPS Recorder	9.0 cm x 5.0 x 1.2, 64 g in air	GPS Location, Satellite Location, Dive behavior	3-9 months	Up to 40
SPOT 5	6.5 cm x 3.5 x 2.0, 42 g in air	Location only satellite tracking	3-9 months	Up to 20
VHF Radio Tags	6.5 cm x 3.2 x 1.0, 27 g in air	Real time detection and location	Up to 1 yr	Up to 40
SMRU GPS/GSM Tags	10.5 cm x 6.5 x 4.0, 84 g in air	Dive behavior and GPS location	3-6 months	Up to 20

Table 4. Types of instruments to be applied to Hawaiian monk seals. Additional instrumentation may be added as new technologies and tags become available. Such instruments would fall within the specifications below and would be used to enhance data collection for the purposes/objectives in this application. Use of new tags not described here will be provided to the Office of Protected Resources.

Instrument Type	Specifications	Application	Approximate Duration of Deployments	# Deployed/Year
		transmitted through cell phone network		
Crittercam Video System	7cm diameter x 20.2 cm long. 1 kg in air 40 g in water	Video Recording of underwater behaviors	3 d – 2 weeks	Up to 20
Video Camera System with additional sensors	Ca. 9 cm long x 9 cm wide x 5 cm high; weight in water ca. 50 g;	Video recording of underwater behaviors; measuring pressure, speed, bearing, temperature, light, acceleration, sound	2 weeks	Up to 20

Health Screening and Instrumentation Mitigation: Mitigation measures described above for handling/tagging seals would apply to any capture activities. The capture team always has a 5-10 minute briefing prior to the event to discuss roles of each team member and contingencies and responsibilities in the event of unanticipated results or action by the animal. Procedures requiring longer restraints such as biological sampling and instrumentation would involve the use of sedatives to calm the animal and reduce stress. Restraint times for normal handling without sedation are very short (less than 10 minutes). Because of this, if an animal is captured for health assessment (or instrumentation) but is not sedated within 8-10 minutes, researchers would discontinue efforts and would release the animal immediately.

A veterinarian is always present when sedation is involved. During an animal's recovery from sedation, vital signs would be monitored, including alertness of eyes, respiratory rate and depth of respiration, and heart rate. In the event of adverse reaction, emergency procedures would be initiated under the advice of an on-site veterinarian. These procedures may vary depending upon the condition of the subject animal, but could proceed in the following order:

1) If respiratory arrest occurs, manual stimulation to restore breathing, including, as necessary, stimulation to face, chest compressions, intubation, and administration of atropine and/or doxapram hydrochloride (Dopram®).

2) If cardio-vascular arrest occurs, administration of epinephrine by the most effective means (IV, IM, pericardial, and/or via airway). Corticosteroids such as dexathmethasone or prednisolone injectable (e.g., Solu-delta Cortef) may be administered after arrest to reduce shock.

3) If the emergency appears to result from diazepam overdose, Flumazenil may be administered to reverse the effects of diazepam.

4) At the discretion of the veterinarian other medications may be administered, including but not limited to sodium bicarbonate, physiological saline, aqueous dextrose solution, and lactated ringer's solution.

Intestinal Parasite treatment (de-worming) with Anti-parasitic Drugs
The deworming (antihelmintic) medicines and routes of administration that may be used include:

Oral Praziquantel and Fenbendazole— Praziquantel Powder (Droncit ®, Bayer, dose 5mg/kg) mixed in an oral nematocide, Fenbendazole paste (Panacur 10% ®, Intervet/Schering-Plough Animal Health, dose 10mg/kg). In August 2009, HMSRP treated 7 juvenile seals (1- and 2-yr-olds) at Laysan Island in this way under Permit 10137. However, this approach was suspended because of difficulties in reliably delivering the entire dose orally in a remote field study. Yet because the oral route delivers the medicine directly to the infected gut, it holds promise as an effective method. HMSRP would like to maintain the option to continue testing the efficacy of this approach. The reliability of oral dosing may be improved, especially with the participation of experienced veterinary technicians and veterinarians, and may be used as an effective treatment in the future.

Injectable Praziquantel (Droncit ®, Bayer, dose 5mg/kg), delivered via intramuscular injection in the dorsal posterior flank, the fleshy region lateral to the spine and inside the line of the iliatic crest. The dose may be divided between two bilateral intramuscular injections to reduce the volume of fluid introduced at any one site. This method was tested on 0-3 yr old seals at Laysan Island in 2009-2010 under Permit 10137. The results were equivocal and further testing is required to determine efficacy and potential positive effects on body growth and survival.

Injectable Ivermectin (Ivomec, dose 0.2mg/ kg) delivered via subcutaneous injection. The injection will be to the loose skin between the shoulder blades (scruff of the neck). Ivermectin is a nematocide and is permitted under Permit 10137, but trials have not been conducted to date.

Topical Profender— Profender is a topical solution that, if effective in monk seals, would allow for deworming treatment with minimal associated disturbance or handling. Profender is indicated for the treatment and control of hookworm infections caused by *Ancylostoma tubaeforme* (adults, immature adults, and fourth stage larvae), roundworm infections caused by *Toxocara cati* (adults and fourth stage larvae), and tapeworm infections caused by *Dipylidium caninum* (adults) and *Taenia taeniaeformis* (adults) in cats 8 weeks of age and older and weighing at least 2.2 lbs (1 kg).

Profender is comprised of two active ingredients: 7.94% Praziquantel and 1.98% Emodepside; its remaining inactive ingredients are hydroxypropionic acid and isopropylidenglycerin. No penetration enhancers are necessary or included in the product for dermal absorption. Profender is a clear yellow ready-to-use solution packaged in single unit dosing applicator tubes for topical (dermal) treatment of cats. Its formulation and dosage schedule is designed to provide a

minimum of 1.36 mg/lb (3 mg/kg) emodepside and 5.45 mg/lb (12 mg/kg) praziquantel based on body weight. For parasitic treatment in cats, the product can be re-applied after 30 days if re-infection occurs. As designed by the inventors of Profender, the preferred doses of emodepside to be effective in a wide range of livestock and domestic animals are from 1 to 20 mg, in particular from 1 to 10 mg, of active compound per kg of body weight (Ventaka-Rangarao, K. & M. Traubel).

Profender solution is absorbed through the skin via the hair follicles, and then enters the bloodstream. Praziquantel travels to the liver and is converted to an active form. Active praziquantel then reaches the intestines and kills tapeworms. Emodepside is transported to the intestine and kills roundworms and hookworms.

Praziquantel has previously been described in detail in Permit 10137 modification requests, thus further description only of Emodepside follows. Emodepside, a semi-synthetic molecule, is a cyclic depsipeptide (Bis-morpholino-cyclo-octadepsipeptide). Emodepside stimulates latrophilin receptor at nematode neuromuscular junctions to signal a transmission cascade that results in a release of inhibitory neuropeptides across the synapse and postsynaptic ion flux, resulting in paralysis and death of the parasite. Maximum concentration of emodepside occurred at 3.2 +/- 2.7 days with a half-life of the drug was 9.2 +/- 3.2 days in cats. Fecal excretion predominates with unchanged emodepside and hydroxylated derivatives as the major excretion products.

General safety studies on emodepside demonstrated a wide margin of safety. On studies conducted on rats that were fed emodepside, the medication was distributed to all organs with the highest concentration in fat. No clinical signs presented in kittens administered Profender for 6 doses at 14-day intervals at 10 times the recommended dose. No fetal or teratogenic impacts occurred when Profender was administered to queens and no adverse effects occurred when it was given at 15, 30 and 50 times the recommended dose to adult cats. In kittens fed topical Profender orally, salivation increased and vomiting occurred in some instances. Emodepside is a substrate for P-glycoprotein. Co-treatment with other drugs that are P-glycoprotein substrates/inhibitors (for example, ivermectin and other antiparasitic macrocyclic lactones, erythromycin, prednisolone and cyclosporine) could give rise to pharmacokinetic drug interactions.

Use of Profender in pinnipeds

Hawaiian Monk Seal—Profender was applied to two captive monk seals. An adult female Hawaiian monk seal (aged 16 years old, 198 kg) held in permanent captivity at Sea World (San Antonio, Texas) that did not carry cestode or nematode infection was treated in November 2009 (L. Dalton per comm.). This seal's treatment with Profender was useful in initially determining any possible obvious adverse reactions that might occur in the species. A dosage of 12mg/kg Praziquantel & 3mg/kg emodepside (0.113 ml/kg)(comprising 20 1.12 ml vials with 85.8mg Profender per ml) was applied topically to the animal's dorsum that had been toweled dry, while the she was held in a temporary holding corral. The animal was kept in the corral (i.e., dry holding) for a period of 1 hour before being allowed access to water. The animal chose to remain out of the water for an additional 3 hours before immersing voluntarily. Observations were conducted constantly for the first hour after Profender administration, and one an hour thereafter for 6 additional hours. No abnormal behavior or adverse reactions (e.g. vomiting,

diarrhea, jaundice, loss of appetite, changes in activity or skin reactions and pelage damage) were observed. A wet spot remained on her pelage where Profender had been applied until the next day.

A juvenile male Hawaiian monk seal (aged 2 years, 90.5 kg) held in captivity at Long Marine Laboratory (Santa Cruz, California) with a prevalent cestode infection was treated in February 2009 (T. Kendall per comm.). A dosage of 12mg/kg Praziquantel and 3mg/kg emodepside (comprising 11 1.12ml vials of Profender) was applied to the animal's dorsum that had been toweled dry, while the he was held in a temporary holding corral. The Profender absorbed almost completely within the first 30 minutes after application. The seal was kept in dry holding 2 hours after treatment. He subsequently chose to not re-enter the water until 1 hour 40 minutes. As with the captive female seal, no adverse reactions were apparent. Examination of cestode eggs in feces collected 30 days post treatment indicated that cestode eggs were still apparent after treatment of one dose of Profender.

Thirteen yearling and 4 two-year old male monk seals at Laysan Island were treated with Profender in June and July 2011. All animals appeared relatively healthy; none were emaciated or wounded at the time of treatment. Each animal's mass was estimated based on comparison of photographs of similarly aged monk seals of known mass. Profender was applied at an approximate dosage of 12mg/kg of Praziquantel and 3mg/kg emodepside to the dorsal pelage. An effort was made to treat seals as they were sleeping, typically some distance up the beach away from the water's edge. None of the animals displayed adverse effects from the medication. A total of 18 post-treatment scats was collected from 7 of the 17 Laysan seals treated; each of the 7 treated seals was sampled 2 to 4 times, 4 to 35 days post-treatment. The 18 vials from treated seals and 13 SAF vials with scat from non-treated seals (i.e. samples from 13 randomly chosen vials from tagged and identifiable seals at Laysan collected in June and July 2011) were examined blind for cestode and nematode egg presence and number via the McMaster egg-counting technique.

42-57% of the treated seals (3-4 of 7) benefited from treatment with Profender. Three seals had zero counts, an additional seal had a large reduction in egg count per gram (epg) to near zero. Only ~8% of the "Controls" (1 of 13) had an absence of eggs (i.e. epg of zero). A temporal pattern in cestode egg eradication in treated seals was not apparent (Logistic regression $R2= 0.003$, $p = 0.77$). Seals had zero egg counts on days 4,14,15,19 and 24 post-treatment (includes 2 zero counts for the animal described above). This trial indicates that Profender is efficacious on some of the seals treated. The prior injectable praziquantel study indicated that praziquantel may improve the condition of animals some of the time (Gobush et al. 2011). Thus, additional treatment of monk seals to better understand its efficacy is likely valuable. Including juveniles from additional sites in future trials may be important because the intensity of infection and response to treatment likely varies on an individual basis, but may also vary temporally and spatially.

Elephant seals

Profender was tested on two juvenile elephant seals (newly weaned seals, approximately 4 months old) being rehabilitated at the Marine Mammal Center (F. Gulland pers. comm.). Profender was applied at an approximate dosage of 12mg/kg to the dorsal pelage while the two

animals were kept in dry holding. None of the animals displayed any adverse effects from the medication. Anti-parasitic effectiveness was not examined because these young animals did not have parasitic infections.

California Sea Lion
Profender was tested on 10 yearling California sea lions being rehabilitated at the Marine Mammal Center (F. Gulland pers comm.). Profender was applied at an approximate dosage of 12mg/kg of Praziquantel and 3mg/kg emodepside to the dorsal pelage. None of the animals displayed any adverse effects from the medication. Anti-parasitic effectiveness was examined for cestodes (*Diphyllobothrium* species) and nematodes (family Anisakidae and *Contracecum* species).

Profender was reasonably effective against cestodes. Of 10 animals tested, all were positive for cestodes before treatment. After treatment, 7 of 10 had 96-100% reduction in fecal egg counts, 1 had 75% reduction, 1 was unchanged (6% increase), and 1 had large increase. Profender was less effective against nematodes. Fecal egg counts before and after treatment were determined, with following results for anisakids: 2 had 100% reduction (from initial low counts), 1 had no anisakids before or after, 7 had increases ranging from 33% to 1000%. Treatment was somewhat more effective against *Contracecum*: 3 had 100% reduction, 1 had 74% reduction, 1 had no change, 1 had a 20% increase, 1 had a 2900% increase, and 3 had no *Contracecum* before or after treatment. Some animals were given access to water; no difference in efficacy was apparent between the animals given water access versus those that were not.

Environmental Effects
The use of emodepside is expected to result in similar or reduced environmental exposure as compared to fenbendazole and ivermectin, two nematocides previously permitted for use in the Hawaiian monk seal. Environmental exposure to emodepside would likely occur via its metabolites' excretion onto land or in the ocean. The potential effects on the environment are summarized as follows.

Chemistry and Fate of Emodepside—Fecal excretion predominates with unchanged emodepside and hydroxylated derivatives as the major excretion products. The substance is not toxic to mammals when tested as a topical application sheep, cattle, horses, dogs and cats. In addition, results of toxicology tests indicate that the Profender is non-irritating to the skin of rabbits, guinea pigs and humans (Bayer 2010). Only negligible amounts of emodepside penetrated through human skin (less than 0.0011% of the applied dose) within a 48-hour time period. Emodepside has low water solubility with a strong affinity to attach to lipids. Therefore when excreted terrestrially in feces it tends to bind to the organic matter in the feces.

Impacts on other species—No ecological data is available by the manufacturer (Bayer) on the effect of emodepside on non-target organisms, other than the mammalian species described above.

Reptiles—A study on reptiles, including aquatic turtles, snakes, geckos, anolids, aganids and varans demonstrated no adverse effects when given at 4 times the dosage (of cats)(Brames, 2008, Schiller et al. 2009).

Fish—There is no data on the effect of emodepside on fish.

Invertebrates—The available data on the toxicity of ivermectin to non-target marine invertebrates is limited to water fleas, Daphnia magna.

Other Biota—There is no data on the effect of emodepside on primary productivity or marine sediment microbes or marine alga.

De-worming Study and Treatment Methods: This activity could involve up to 300 seals annually, but during the study phase (to establish efficacious deworming methods), the number of seals per year is estimated not to exceed 100.

HMSRP researchers would identify study subjects during standard ground surveys and would assess health status and body condition by visual inspection and examination of digital photos. HMSRP uses body condition indices to subjectively score seals as good, medium, thin, or emaciated; indices are based primarily on visibility of the pelvic girdle, ribs, point of the shoulder, peanut head, and vertebrae. Emaciated seals too compromised to treat without high risk of mortality would be excluded from the research study. For future enhancement treatments, once the technique has been deemed effective (evidenced by improved body condition or survival) and of low risk to seals based on results of the research study, HMSRP would attempt to treat seals in the least invasive manner (i.e., not capturing seals for assessments and sampling). Any seal (healthy to emaciated but not moribund) may be treated in the future after completion of the study.

For research, seals would be randomly assigned to a treatment or control group, or alternated systematically. The goal, to the extent feasible, is to have equal numbers in each group, matched in age, sex, body condition, and location. Sex matching is important because sex has been recognized to influence worm burden and its effects on the host in other mammals (Wilson and Moore 2002).

The study will likely be conducted at multiple sites and years, which is necessary due to a high degree of variability in juvenile monk seal survival both temporally and spatially (Baker and Thompson 2006) and the small number of juveniles available in any given year at any one site.

Treatment with oral or injectable drugs—Hawaiian monk seals would be handled for treatment (i.e., administration of oral or injectable worming medication) up to four times per year and follow up assessment (i.e., sampling and weighing) also up to four times per year. Thus, maximum number of takes per seal per year would be eight. However, whenever feasible, follow up assessment would be combined with subsequent treatments in a single capture to reduce the number of takes. Seals age 1-3 years would be treated during any of these times. Pups would not be treated unless they have been weaned for at least 120 days. HMSRP epidemiological sampling conducted from 1998-2000 (n=54 for weaned pups) indicated that 100% of pups sampled who were at least 120 days post-weaning (n=15) tested positive for tape worms or round worms. Conversely, only one pup sampled at less than 75 days post-weaning (n=39) tested positive. Treatment of pups >120 days post weaning would ensure that most would be infected.

All study subjects are captured by hand and net. Feces may be collected for subsequent determination of parasite burden/presence (voided feces or fecal sample collected via fecal loop or digital extraction; stored in 10 % formalin). Seals would be measured and weighed, ultrasound performed, flipper tagged if necessary, and treated with the appropriate dosage for the particular medication(s) being tested as specified above, then released.

Fecal sampling would be used to confirm presence of parasites. Control seals would be handled and sampled in the same manner as treatment seals without receiving any injection.

No sedation would be required for either treatment or control seals. However, the study may be facilitated by conducting it in conjunction with other research involving capture and handling of juvenile monk seals (e.g., foraging and health assessment studies), in which case protocols may involve sedation, biomedical sampling, and instrumentation.

HMSRP would attempt to re-treat/capture all study subjects (same protocol as for initial treatment) during subsequent field sessions throughout the year (at approximately 7-12 week intervals) and over subsequent years. Multiple treatments would be intended to clear adult worms that survive treatment (or are acquired thereafter) and migrating larva that mature after treatment. Controls would be handled at the same frequency.

Post treatment body condition and fecal egg counts would be determined by observing the seals, collecting scat from known individuals during HMSRP monitoring patrols, and capturing, weighing, and conducting ultrasound on seals. Visual assessment of condition would be recorded on an ongoing basis throughout the study, using standard HMSRP subjective body condition scoring and feces would be preserved for detection of parasites.

Subsequent survival would be determined through visual re-identification during population assessment field research, supplemented by observations made during additional field sessions. The duration of the survival period would be dependent upon the timing of the initial field phase of the study relative to the assessment field season. That is, at those sites lacking a constant field presence, seals that are not observed in the subsequent field session may have died at anytime during the interim from the previous observation. The resolution of the survival assessment is therefore limited by the frequency of the field presence.

The primary statistical analysis would consist of modeling survival (either with capture-recapture or logistic regression) of treatment and control animals to determine whether there is evidence that anti-helminth treatment improves survival. Other factors that influence survival (e.g., predation) would be treated as covariates. Other important analyses would be a comparison of body condition change in treated versus control animals using both quantitative and qualitative measures, and the comparison of parasite loads in control versus treatment seals (see Gobush et al 2011).

Treatment with topical Profender— Efficacy test. The efficacy of Profender to kill adult helminthes in juvenile Hawaiian monk seals would be tested on a sample of juvenile seals anywhere in the Hawaiian Islands. As described above, some efficacy investigation has been

conducted at Laysan Island in 2011, indicating about half the treated seals had cestode infections essentially cleared with a single application of Profender, though the sample size was small. This study needs to be continued to better characterize the efficacy of Profender. Although Profender is designed to kill both cestodes and nematodes in domestic cats, HMSRP is primarily interested in treating cestode infection with Profender because these parasites have significant impact on condition in Hawaiian monk seals (Reif et al. 2006). Also, a trial of Profender on captive California sea lions (*Zalophus californianus*) indicates that it is highly effective in killing cestodes (96-100% reduction in 7 of 10 individuals) but not nematodes (100% reduction of *Anaskarids* in 2 of 9 individuals, and a 100% reduction of *Contracecum* in 4 of 7 individuals) in pinnipeds.

Up to 60 seals of either sex aged, aged yearling to three years old would be treated and post-treatment beach scats would be collected from as many of these individuals as possible. To minimize handling, we will attempt to apply the Profender while seals are sleeping without causing any disturbance. This is analogous to application of pelage bleach, which is typically done without waking seals. Trial applications of Profender in 2011 showed that the drug can be reliably applied without disturbance. If seals awaken during application, they may be briefly restrained to the degree necessary (likely less than 1 minute) to apply the Profender.

Seal mass will be estimated visually (based on experience with similarly-sized seals that have been weighed) prior to treatment and transfer the appropriate amount of solution from the manufacturer's packaging (1.12ml ampoules) to a 5 or 10cc syringe for easier application. Application of Profender will consist of locating the region of the upper dorsum (e.g. between the scapulae), ensuring that it is basically dry, clean and free of infections, lesions or recent wounds. The solution will not be applied beyond the upper dorsum to ensure that the seal cannot touch with its fore flippers or bite with its mouth the area of application after treatment. We will place the tip of the syringe into the seal's fur, and depositing the medication. The target dosage will be 12mg/kg of praziquantel and 3 mg/kg of emodepside; for a 90kg seal this will be approximately 11 of the manufacturer's ampoules of Profender. The safety margin of Profender is wide; cats that were dosed up to 50 times the recommended dosage displayed no adverse effects.

If capture and complete restraint is required for application (e.g., if combined with other procedures), we will treat the seal, then cool the seals' hind flippers with seawater prior to release to aid thermoregulation. In addition, we will attempt to direct seals away from the water for up to 30 minutes after application of the medication by herding them toward higher ground if necessary or placing deterring objects, such as plastic mesh net or other such material between seals and the water as a passive deterrent. The minimum required coercion would be used. If the seal is above the beach berm, researchers will remain out of sight below unless pressure is required to prevent the seal entering the sea. If a seals displays signs of undue distress, agitation or fail to become calmer after application, we will allow the animal to proceed into the water and will cease deterrent methods. Procedure for monitoring will be similar to that previously described; additional notes will be recorded of how the seal behaved following application of Profender and how soon after treatment it entered the water (up to one hour post-treatment).

Procedure for collecting scat will be similar to previously described (fecal loop or digital fecal collection). We assume that seals included in the efficacy test are infected with cestodes because previous sampling found the infection rate was 98.5% (66 samples, 39 unique juvenile seals). However, an effort will be made to collect feces from treated seals from the beach without disturbance prior to application of Profender. Thereafter we will collect beach scats from all treated seals for the remainder of the field season. Though a control group will not be included in this test, we will attempt to collect beach scats from untreated 1-year-old seals (males and females) during the period of time that we are conducting the efficacy test.

Procedure for determining helminth egg presence will be as previously described. Cestode egg presence or absence in scats collected from 3 days to up to 4 weeks post-capture will be used to determine the efficacy of Profender. Following that period, egg presence will help determine time to re-infection when to potentially time retreatment in future trials. Examining egg presence, especially of Anaskarids and *Contracecum* species, in scats of untreated seals will provide information on infection rates of juvenile monk seals at each study site.

Because Profender was effective in 42-57% of the free-ranging seals treated in 2011, some seals may be treated at the target dose (e.g. 12mg/kg praziquantel) applied as two consecutive treatments, spaced 14 days apart, to test if repeated dosing on a short interval improves its efficacy. Alternatively a single treatment of an increased dose of 15mg/kg praziquantel and 3.75 mg/kg emodepside may occur. The same or other juvenile seals may be treated. Additional treatment rounds will only occur at least 14 days from the previous treatment.

Tests of the relationship of reduction in cestode burden, condition and survival—If the results of the efficacy tests demonstrate that Profender reliably kills tapeworms, as evident by an absence of cestode eggs or a reduction in eggs to near zero in scats collected up to four weeks after 1-2 consecutive treatments (spaced 14 days apart) from a high percentage of animals tested across sites, then we may test the hypothesis that intermittent reduction of parasite load improves condition and survivorship of juvenile Hawaiian monk seals. Less than 200 juvenile seals would be treated in this study. Criteria for inclusion, assignment of treatment groups, handling, restraint, weighing, release and monitoring procedures, analysis and evaluation of results will be similar to that described previously with inclusion of the medication application and post-release details described here. However, capture and restraint will only be necessary when weighing occurs, if it proves feasible to apply the medication on unrestrained seals. If light restraint for application of Profender is needed (see description above), the animal will be directed away from the water as described above for up to 30 minutes after application. Subsequently the seal may be briefly recaptured for weighing. This sequence is intended to minimize stress as the medication absorbs. Otherwise (when weighing does not occur), seals will be restrained sufficient to apply the Profender and we will attempt to keep them on shore for up to 30 minutes as described above.

Treatment will occur up to 12 times per year for 1- to 3-year-old seals, if the topical medication can be applied with no disturbance or minor disturbance. If capture and restraint are required then treatment would only occur 4 times per year. The minimum treatment interval will be consistent with the re-infection rate demonstrated by fecal cestode egg presence in the efficacy tests. Weighing at the time of treatment, which requires capture and restraint, will occur up to 4

times per annum for 1- to 3-year-old seals. Young-of –the year will enter the study once they are at least 120 days old; therefore the number of times they are treated and weighed per annum may be less than that of the older juveniles. The minimum effective dosage of Profender will be used (either 12 mg/kg or 15 mg/kg). Seals treated in the efficacy test(s) will maintain their treatment status.

De-worming Treatment Mitigation: Mitigation described for activities requiring handling and restraint would apply to this action. Seals would be monitored by follow up assessments as describe to determine the effects (positive or negative) of worming treatments. If there is any indication that the welfare of the subjects has been compromised by handling, treatment, or any other artifact of the study, HMSRP would cease treatments and would consult with the Monk Seal Health Care Working Group to evaluate the preliminary results. Previous testing of injectable Praziquantel resulted in three cases of swelling at the injection site (all of which resolved) and one case of an abscess at the injection site (which was treated by field staff and resolved). Improved handling methods, re-doubled vigilance in cleaning the injection sites and maintaining the cleanliness of needles, syringes and medicine, and splitting doses of Praziquantel were subsequently employed, after which no further swellings or injections occurred. These procedures would be followed in future and field staff would be prepared to treat any abscesses should they appear. The use of Profender is intended as a mitigation of disturbance and risk of treatment, as it is a topical solution that requires minimal or no restraint to apply.

Translocation Methods—Enhancement and Research

Translocation Methods: Five types of activities would occur, as described below. Seals may be taken more than once by any of the plans. In particular, attempts to unite abandoned pups with a prospective foster mother may occur up to three times or more with the same prospective mother. Translocations to remove an animal away from hazardous areas may need to be conducted more than once if the animal subsequently moves to hazardous sites.

1) <u>Establish/reestablish maternal association</u>: Annually, as warranted (estimated up to 20) nursing pups of either sex that have become prematurely separated from their mothers may be relocated annually to either reunite them with their mother or to another parturient adult female seal which has lost her pup (primarily in NWHI but as necessary at any location in the Hawaiian Archipelago). Abandoned nursing or prematurely weaned pups would be captured by hand or with a hoop net or stretcher net without sedation, and carried to a parturient female. The transport distance would usually be very short, generally no more than several hundred yards, so transport time would not exceed 30 minutes. In rare instances, intra-atoll transport via small boat may be necessary if the parturient female is located on a different islet from that on which the pup was abandoned. In such cases, the pup is secured by the net in the boat. No holding in a temporary enclosure would occur. The same methods would be used to reverse a pup exchange that has disadvantaged a female pup in favor of a male pup. The only difference being that the two pups would be captured from the nursing mothers and returned to their original pairing. In the case where a previously weaned male pup has usurped the nursing position of a female pup, the male would be capture and carried a sufficient distance away and placed in a safe location.

2) Risk alleviation: Annually, as warranted (estimated up to 60) pups of either sex which have weaned or are determined to be very close to weaning (e.g., a few days prior to weaning) in locations here there is a severely reduced chances of survival, such as areas of high shark predation e.g., at French Frigate Shoals) or likelihood of human/fishery interaction (i.e., in the MHI), would be moved to other beaches or islets which present less risk. Pups born within the NWHI would be moved to other sites within the same NWHI atoll, and pups born within the MHI would be moved to other beaches or islands in the MHI.

Weaned pups in high-risk areas would be captured using a hoop net or stretcher net, without sedation. In the NWHI, pups that wean in high-risk areas would be captured with a net and transported via small boat directly from weaning site to release site within the same atoll or on the same island. This typically occurs at French Frigate Shoals, transporting from Trig Is. to Tern or another safe island, but could occur at any location in the NWHI where weaned pups are subject to severe and immediate risks of death.

In the MHI, weaned pups in danger of high human contact (and with the potential for exposure to disease, harm, fishery interaction, or habituation to humans), transport would be in a cage on a vehicle (truck), boat, plane, or helicopter, and the duration of transport would generally not exceed 8 hours. Pups would be transported immediately after capture, and no temporary holding in a pen would occur unless deemed necessary by an attending veterinarian (e.g., for health screening).

Weaned pups translocated in the MHI may be health screened by an attending veterinarian (as described above), and may be instrumented (VHF and/or satellite or cell phone GPS tags, as described in Table 4 above) would be deployed to monitor the seals' movements and foraging behavior post-release. Most animals transported in the NWHI would not be sampled, because the translocations would be of short duration, and a veterinarian is routinely not on any NWHI site.

Other activities under this category would include but are not limited to cutting an umbilicus from a neonate pup that is being encumbered by a placenta, hazing seals of any age/sex away from other harmful situations (e.g., roads or boat ramps), and removing seals of any age/sex from behind the Tern Island seawall at French Frigate Shoals where they could be trapped.

3) Survival enhancement, 1st stage of two-stage translocation: Up to 20 healthy weaned pups and juvenile seals of either sex that have weaned at subpopulations where juvenile survival is low may be relocated annually to other subpopulations with higher rates of juvenile survival. First stage weaned pup translocations may occur within the NWHI, from the MHI to the NWHI, ***but not*** between the NWHI and MHI.

A decision framework for determining the source subpopulations, number of weaned pups (not to exceed 20 per year) and recipient subpopulations is described in detail in Appendix A (two-stage translocation). Many of the inputs to the decision framework rely on direct observation of key indicators such as population status, juvenile survival rates, and outcomes from previous translocation actions. Also, at various points in the decision framework, researchers would use a

computer model (called a stochastic simulation model) updated with the most recent seal population data to estimate the likely range of benefits associated with different choices.

Two decision trees, one for each of the two stages of the translocation strategy, have been developed to support decision-making and assessment as translocation projects progress. The *Stage 1* decision tree addresses translocation of weaned Hawaiian monk seal pups from areas of lower survival to areas of higher survival. The *Stage 2* decision tree addresses returning previously translocated seals from the recipient site to their donor sites. The decision framework is described in detail in Appendix A and is briefly characterized below.

The decision framework is designed to structure the decision making process so as to maximize the benefits and reduce the risks associated with the translocation project. For example, the donor and recipient sites will be carefully chosen to achieve the greatest possible benefit (in terms of increasing juvenile survival and enhancing population welfare), while ensuring that the capacity of a site to support additional monk seals is not exceeded. This will be determined from observations of juvenile survival at each site, supplemented by simulation modeling to better quantify the probable benefit.

There are provisions within the framework to suspend translocation actions in response to unforeseen developments such as the failure to return previously translocated seals to their natal site or region once they reach the stipulated age. While seals are in the wild at the recipient site, they will be monitored (initially with satellite transmitters, and later through the regular population assessment program) so that researchers know as much as possible about their location, activities and welfare.

Proper care and safe transport of seals as well as mitigating risks of transmitting disease via translocations are other important considerations that the HMSRP has accounted for. Details of the measures involved in selection, health screen, care in temporary captivity, quarantine and unforeseen contingencies are addressed in Appendix F (Health Screening and Quarantine). The HMSRP has decades of experience handling and transporting monk seals (Baker et al. 2011), especially weaned pups, and best practices developed to date are to be employed. As new information accrues during the implementation of future translocations, this will augment and help refine protocols further.

As envisioned, the translocation project will initially be implemented as a small-scale experiment. The first phase may involve the experimental translocation of juvenile seals from one site to another to better assess how well the second stage of the translocation will proceed. As the project proceeds, results from the preceding actions will be used to inform future efforts and better predict the expected outcome from each candidate action. For example, researchers are particularly interested in knowing how different the survival of translocated seals will be relative to those that have spent their entire lives at a site. Once there are data with which to assess that difference, it will be used to better refine the predictions from the simulation model.

4) <u>Survival enhancement, 2st stage of two-stage translocation</u>: Up to 30 juvenile or sub-adult monk seals may be translocated annually. This action constitutes the second stage of two-stage translocation and the methods are congruent with those described in the first stage, with details

provided in Appendices A and D. The reason why the number of annual takes (30) for the second stage exceeds that for the first stage (20), is that the return (second stage) translocation may occur any time after surviving seals from the 1st stage have reached age 2 or 3 yr. If some seals are not captured and returned by age 3 yr, they would be subject to return in subsequent years. In this way, the number of seals eligible for return translocation may accrue over time.

5) Experimental translocation for research: As described in the Project Purpose section, a small number of seals (maximum of 6 per year of any sex and including juveniles, subadults, and adults) may be translocated in order to better anticipate the results of the second stage of two-stage translocation. The methods would be the same as for translocation of juveniles and sub-adults as described in the preceding section, and Appendices A and F. This is a research project, but it may be that translocation of seals with unmanageable behavior (human interactions) in the MHI could provide part of the information that this research is meant to yield. If so, such unmanageable seals would be translocated, thereby achieving both an enhancement action (in that these seals would otherwise not likely be able to remain in the wild population) and obtaining research information with a single take. Alternatively, juvenile and sub-adult seals could be translocated to different subpopulations within the NWHI.

Translocation Mitigation: Appendices A (Translocation) and F (Health Screening and Quarantine) describe how translocations will be carried out, and a major focus of those documents is mitigation, primarily minimizing the probability of undesirable outcomes. Some of the outcomes the decision framework and protocols are designed to avoid include, but are not limited to:

- Harm or illness to animals in capture or transport
- Introduction or spread of infectious disease
- Reduced chance of survival of translocated seals
- Reduced fitness of individuals in the recipient subpopulation
- Imbalanced population sex ratios
- Depletion of donor subpopulations

Follow up monitoring at the individual level (i.e., of translocated seals via telemetry and visual surveys) as well as at the subpopulation level (population monitoring) will provide information for project evaluation and help improve future translocation decision making.

Enhancement – Adult Male Removal Methods

Adult Male Removal Methods: We will remove adult male seals in cases where the individual seals are known or strongly suspected of causing serious injury or mortality of conspecifics as a result of single or multiple male aggression. Removal may include translocation to sites within the Hawaiian Islands or Johnston Atoll capture for permanent maintenance in captivity, or, as a last resort, euthanasia. We request permission to remove adult male seals in cases where the individual seals are known or strongly suspected of causing serious injury or mortality of conspecifics as a result of single or multiple male aggression. In some cases, researchers may be able to stop the attacks and treat injured seals, but the aberrant behavior continues. Euthanasia would be considered in cases where the identity of male aggressors is certain or circumstantial evidence overwhelmingly indicates direct participation in such attacks. Adult males will be taken

by capture, restraint, sedation, biological sampling, instrumentation, and transport; or, humane euthanasia. Up to 20 adult male seal seals may be taken annually, with a limit of 10 lethal takes over the 5-year requested duration of the permit.

When adult male aggression appears to be occurring as indicated by disappearance of weaned pups, wounding or death of seals with injuries consistent with male attacks, field crews focus observation effort in order to identify the males involved. Because single male aggression tends to be directed mostly at weaned pups, which spend much time on the beach, identifying the males involved is somewhat tractable. Even so, there are cases where males are documented habitually behaving aggressively towards pups but unequivocal documentation of an aggressive act that causes a death or severe wound are lacking.

It is far more difficult to ascertain perpetrators of multiple male aggression. Most mating behavior occurs far out to sea and is thus rarely observed. Still, males compete over the right to lie next to, and maintain exclusive access to, females on shore. It seems likely that males that are successful maintaining exclusive access to females on shore are also more successful maintaining exclusive access to females during mating events at sea. To reduce the risk of increasing aggression by inadvertently removing males that maintain social control, the relationship between aggression and apparent dominance was investigated prior to undertaking the male removal efforts during 1984-1994 (Johanos, et al. 2010) and the HMSRP continues to collect behavioral data relevant to this question. Most males are never seen on the beach beside a female who is in, or near, estrus. However, a small subset of males commonly associates with these females (i.e., lie next to them on the beach), successfully defending them from other males, and are considered dominant. At Laysan and Lisianski Islands, where multiple male aggression has been most common historically, field technicians document the identities of paired adult males and females.

All adult males in a population can be ranked according to the proportion of observed pairings of males with adult females at or near estrus in which each male was involved (Johanos, et al. 2010). For example, a male who accounts for 10% of all observed pairings with adult females near estrus is considered higher rank than a male that accounts for 2% of these pairings. This dominance ranking is informative because past analysis has shown that subordinate males are most likely to be involved in multiple male aggression. Although identified aggressors are typically subordinate, there is usually at least one dominant male present in an aggressive aggregation. Typically, that male would have been already paired with the female when the aggressive incident began. Anecdotal observations suggest that the risk of aggression may be higher for females paired with lower-ranking males within the dominant group than for females paired with the highest-ranking males (Johanos, et al. 2010).

When there is evidence that multiple male aggression is occurring (typically due to observation of severely wounded or killed adult females), field technicians increase effort to observe actual aggressive interactions or aggregations of many males with a wounded female. Three types of cases could result in a decision to remove some adult males:

1) If a multiple male attack is observed in which a victim is seriously wounded, and males involved are identified, these males may be removed. However, the highest dominance-ranked males thus observed, especially those previously observed attending the victim on

the beach, would most likely not be considered for removal unless they were specifically observed causing a serious injury to a female seal.

2) If an aggregation of several identified males is observed either
    a. associated with a freshly wounded victim (i.e., the aggression is not observed but it is clear that it very recently occurred);
    b. involved in competition for a victim but the victim is not seriously harmed (i.e., sustains only normal mating injuries); but this observation occurs during a period when other seals have been observed with male aggression injuries, then these identified males may be removed. Again, the highest rank males identified in such an aggregation (or the previously observed attending male) would not be removed.
3) Finally, if unusually high levels of male aggression are occurring yet no males can be confirmed as aggressors nor suspected based upon observations such as 2a or 2b above, then some low ranking males that are observed consistently in the same beach areas as victims may be considered for removal.

If the HMSRP decides to remove adult male seals, the arrangements for the disposition of the seals in question will be completed before the action is taken. That is, transport via vessel or aircraft will only occur after a permanent captivity site, translocation destination, or lethal removal has been determined.

1. *Permanent Captivity*: Seals will be captured and restrained using a hoop net or a throw net. Diazepam or midazolam may be administered for sedation at the discretion of an attending veterinarian. Atropine may be administered as a pre-medication, or as an emergency drug for treatment of certain cardiovascular emergencies and complications (e.g., bradycardia), based upon the discretion and clinical judgment of the on-site (attending) veterinarian. Seals will then be transported either to a temporary holding pen or directly to a transport vessel. Local transport will be via either small boat or over land, as appropriate to the location, and will generally not exceed 2 hours. Ideally the seal will be captured at a beach that is accessible by small boat. The animal will be captured, placed into a cage, and the cage carried by hand (requiring at least 6 people) to the nearby small boat. For adult seals some form of land transport such as a portable cart may also be used. If transport over land is not feasible, a seal would not be captured unless it is on a beach accessible by small boat for transport. The animals will be kept wet throughout the duration of transport.

    The feasibility of constructing and using a temporary holding pen varies at different sites within the NWHI. At present, staff and supplies are available for such an endeavor only at French Frigate Shoals and Midway Atoll. Temporarily holding adult seals in a pen at other islands can be accomplished only if sufficient staff (for adult seal restraint and pen construction and maintenance) and construction materials are located at the appropriate site. The primary structure for temporary holding (longer than approximately two days) will be shoreline pens measuring up to approximately 24 ft x 80 ft. Approximately 30% of the surface area will include water at least 2 ft deep at lowest tide. The remainder of the pen will be intertidal and dry resting area above the high water line. No more than 5 seals would be held in a pen at any one time. In some instances requiring short,

temporary captivity (e.g., less than two days), a shaded holding pen may be erected in the vicinity of the field station, and seals would be wetted down periodically.

It is not practical or efficient to have sufficient personnel and equipment stationed at all six major Hawaiian monk seal NWHI breeding sites to anticipate conducting an action, which may not prove necessary. At such times and locations when we have advance documentation of aggressive males, we will be able to devote sufficient resources to consider temporarily holding adult males in a pen at the site. However, if action is required on short notice at a site, which lacks additional personnel and pen materials, we will, if feasible, dispatch a vessel with transport cages and capture personnel. Seals will be transported directly to the vessel and will not be temporarily held.

Seals placed in a temporary holding pen will remain there until a vessel is available to transport them to Oahu. This length of time will vary, but should not exceed two weeks. If availability of a vessel within two weeks is not assured at the outset, seals will not be captured. In the event that seals are captured and placed in a pen, but circumstances delay arrival of the transport vessel, the condition of seals will be evaluated and other options for removal will be considered. Seals in the temporary pen will have both shallow water and haulout space available to them. If seals are held longer than 4 days in the pen, they will be offered herring.

Seals may be again sedated with diazepam prior to their transport from the holding pen to the transport vessel. Adult monk seals must be sedated in order to safely put them into cages. Herding unsedated animals into cages is difficult and exceedingly stressful to the seals. Sedated animals are more tractable, yet are not immobilized, so they can still be safely herded/pushed into cages by handlers. During transport on the vessel, seals will be maintained in individual cages and kept wet during daylight hours. Transport to Honolulu aboard the vessel will not exceed one week. Seals will be offered herring daily on the ship during transport.

Upon arrival in Honolulu, seals will be placed in approved temporary holding facilities (e.g., the new NMFS Ford Island Facility, the Waikiki Aquarium, or the planned Marine Mammal Center's captive facility on the island of Hawaii). If other seals are being held in these facilities at the same time, strict quarantine procedures will be followed. Seals will remain in temporary holding until arrangements are made to transfer them to a permanent facility. As stated above, seals would not be brought into Honolulu unless a site had been identified as willing to take the seals, and willing to obtain the necessary permits to accommodate them. Details of captive holding are provided in the Captive section below.

2. *Relocation of adult males:* Adult males would be treated identically to those taken for permanent captivity (above) except for their final destination. Seals would be transported to Johnston Atoll or elsewhere in the Hawaiian Islands for release. Animals may also be health screened in accordance with procedures listed above if a veterinarian is present. Prior to release, each animal will be sedated and fitted with a satellite transmitter so that the animals' positions can be determined. This will provide information on the fate and

destination of the seals, should they move away from the release site. The transmitter will be operational for up to one year, although, depending on the date of capture, the duration of instrumentation may be shorter due to the seals' annual molt.

Seals may be taken more than once if they return to the site from which they were removed and resume aggressive behavior against immature seals.

3. *Lethal removal of adult males:* If neither option described above is available, seals will be humanely euthanized. Seals will be captured, sedated, and sampled as above. Thereafter, seals will be injected with a lethal dose of Beuthanasia® (sodium pentobarbital) into the extradural vein. At least 60 ml (lethal dose 1 ml/10 lb) will be injected. Immediately after the animal has succumbed, a complete necropsy will be conducted, with samples saved from all major organs. Because of the presence of barbiturates in the carcasses, all soft parts not retained will be collected in plastic bags for subsequent environmentally safe disposal (e.g. incineration). This requires carcasses to be removed from the NWHI.

   In addition to injectable sodium pentobarbital, the HMSRP requests permission to have additional modes of euthanasia available (penetrating captive bolt and gunshot). The following information was obtained from the American Veterinary Medical Association Guidelines on Euthanasia (AVMA 2007). Penetrating captive bolt is commonly used for euthanasia of domestic animals (e.g., cows, horses, swine, dogs) and causes concussion and trauma to the cerebral hemisphere and brainstem. Seals would be sedated with valium (IV) or midazolam (intramuscular; IM) and restrained for use of the captive bolt gun. Euthanasia by this method would be carried out by an experienced veterinarian or qualified field researcher under the consultation of a veterinarian.

   Gunshot is another requested form of euthanasia. A properly placed gunshot can cause immediate insensibility and humane death. In some circumstances, to minimize stress from capture and restraint, gunshot may be the most practical method of euthanasia of wild, free-ranging species. Only highly skilled personnel trained in the use of firearms should perform such euthanasia. Also, shooting should only be performed in jurisdictions that allow for legal firearm use.

   Euthanasia of the same species under different conditions may require different techniques. A veterinarian experienced with Hawaiian monk seals would be consulted when selecting the method of euthanasia, in accordance with AVMA guidelines (AVMA 2007).

Hazing adult males: Aggressive adult male seals may be hazed away from conspecific victims under two circumstances:

1) When the victim (typically a weaned pup) is in imminent danger of serious injury or death. For example, males may exhibit aggression towards pups but the pup may escape or the male may not act overly aggressive. However, at times, males mount and hold pups underwater until they are at risk of drowning. Or they may bite them severely. In such cases, field staff may haze the male away from his victim.
2) When the aggressor has previously been observed to harm or kill a conspecific. For

example, a male may have met the criteria for removal (above), yet logistics and cost constraints may delay that action. In the interim, the repeat aggressor may be hazed away from any potential victim (typically a pup or juvenile) he is approaching or attacking.

Hazing aggressive adult male seals may be accomplished by approaching, vocalizing or otherwise making noise, prodding with a long pole, or throwing objects (e.g., rocks, coral, sticks, debris). Care will be taken not to harm or cause severe pain to the male. The objective is to distract the target animal and frighten him away rather than to cause harm or pain. However, the risk of death to a conspecific outweighs any risk of injury to the adult male.

Adult Male Removal Mitigation: For translocation and permanent captivity, seals would be captured, temporarily held and transported using the methods and restrictions described above to minimize stress and risk to the seals' safety. This includes the use of sedation to reduce stress during handling. Transported seals would be kept wet throughout all daylight hours, but would be otherwise undisturbed. Procedures for responding to emergencies to mitigate negative impacts from restraint or sedation would be followed as described above for health assessment sampling. If seals are euthanized, AVMA-approved methods would be used in consultation with an attending or consulting veterinarian.

Enhancement – Disentanglement and Dehooking

Disentanglement and Dehooking Methods: HMSRP biologists would release Hawaiian monk seals that have become entangled in marine debris, or remove fishhooks from monk seals that have become hooked, to increase their chances for survival. As warranted, an unlimited number of seals of any size or sex would be taken annually from any site in the Hawaiian Archipelago, but typically this would not exceed 75 animals annually.

Seals which are observed to be entangled by nets, lines, or other marine debris would be freed via two possible methods: (1) Animals would be captured with a hoop net or a stretcher net, restrained, disentangled (by hand or by using a cutting implement), and freed; or (2) The entangling item would be cut free using a cutting implement attached to a long pole, with no attempted restraint of the animal. The choice/use of each technique would be at the discretion of field personnel performing the rescue. If a line or net fragment has become embedded in the tissue of the animal, the entangling item would be removed as carefully as possible to avoid further injury to the seal. This may require cutting the item at several sites to avoid pulling net knots out through tissue. Betadine® or similar disinfectants may be applied if warranted and available on site. Seals that are released may be either on the beach or on a near shore reef.

Hooks would be removed from seals by restraining the animal in a hoop net or stretcher net and removing the hook by hand. The animal may be sedated if necessary to ensure the safety of responding personnel. Sedation procedures would be identical to those described for health screening above, and would be done by a veterinarian. Any entanglements in the MHI to which HMSRP responds would be coordinated with the stranding coordinator at PIRO.

Field camps with boats (French Frigate Shoals, Pearl and Hermes Reef, Midway and Kure Atolls, and occasionally Laysan Is.) also remove debris from marine habitats when possible and

have located and released seals entangled offshore. All occurrences of entangled individuals are recorded, including seals with fresh entanglement scars that were not previously observed. The entangling gear, or a sample thereof, is also collected and catalogued. Data are provided to the NMFS Stranding Coordinator at PIRO.

Disentanglement and Dehooking Mitigation: Disentangling seals and removing hooks is a form of mitigation to prevent serious injury and death to monk seals. Mitigation employed to minimize impacts to subject seals used during other monitoring/capture/handling events applies here. Biologists would attempt to remove entangling items without restraint (i.e., by using a long-handled cutting implement) whenever possible. If restraint or sedation is necessary, seals would be cooled with water if restraint is not near the waterline and is during midday times.

Necropsy Dead Seals

Necropsy Methods: HMSRP would conduct necropsies on carcasses of Hawaiian monk seals found dead in the Hawaiian Archipelago. Responses to dead seals in the MHI are coordinated with the stranding coordinator at PIRO. No take of live animals is involved, solely retention of marine mammal parts for determination of cause of death. The number of necropsies to be conducted is unlimited (i.e., as warranted).

Carcasses of seals would be necropsied in manner normal for phocids and specific to Hawaiian monk seals (Winchell 1990) and protocols defined in Appendix G (Necropsy Sampling), which specifies samples to be taken, appropriate method of sample storage, and sample analyses. Such analyses may evolve as new tests are developed and disease risks are encountered. Specimens would be retained according to the condition of the carcass. If the animal has recently died and the carcass is in good condition, samples from all major organs would be retained and life history and morphometric data recorded. If the carcass is in poor condition, a limited set of data would be collected, including size (measurements), sex, and general description. Skulls would be retained for subsequent measurement and additional skeletal materials may be retained. Necropsy data are given to the stranding coordinator at PIRO for completion of NMFS stranding forms.

Salvaged parts may be used to enhance survival of pups (after necropsy, seal tissue may be used as bait for permitted shark removals).

Opportunistic Sample Collection

Opportunistic Sample Collection Methods: HMSRP would opportunistically collect specimens, including placentae, scats/spews, and molted fur/skin, at haulout sites of Hawaiian monk seals. No marine mammals would be directly taken during the specimen collection, but some animals may be incidentally harassed. Placentae are collected to be examined for possible causes of perinatal death; scats/spews are collected for dietary analyses; molt/skin would be collected and retained for DNA isolation.

Placentae would be collected from pups which are stillborn, or which experienced perinatal death. Many of these placentae would still be attached to the carcasses of the pups (and would be therefore collected as part of necropsies), but some may be separated from the pup,

particularly if the pup was alive for a short time after birth or if the pup carcass has been washed to sea.

Retrieval of most scats, spews, or molted fur/skin samples would occur after seals have departed the haulout locations to forage at sea, leaving behind samples of interest. In some instances the identity of the seal that left the sample or item would be determined if tracks are evident between a seal still on the beach and the item to be collected.

Preservation methods for all specimen items would be as described in Appendix G. Necropsy specimens and subsamples of placentae would be fixed in formalin for histopathological analyses, and the entire remaining placenta would be frozen. Molt/skin samples would be collected and kept dry. Some necropsy samples are frozen in liquid nitrogen. Scats would be stored in a solution of detergent/seawater, although subsamples of fresh scats would be preserved in PVA (polyvinyl alcohol) to be examined for parasite eggs. Whole parasites from scats and spews would be preserved in AFA (alcohol, formalin, acetic acid–for cestodes and trematodes) or AG (alcohol, glycerin–for nematodes). Other items from spews (bones, otoliths, scales, beaks, etc.) would be preserved in ethanol, although fresh flesh from spews may be frozen for ciguatera analyses.

Necropsy and Opportunistic Sample Collection Mitigation: Researchers would reduce disturbance as discussed in mitigation measures for monitoring and other activities. Sample collections would not be made if seals are in the immediate vicinity and would likely to be disturbed by the activity. No placentae would be collected if the birth mother is still in the immediate vicinity and liable to be disturbed. Carcasses would be moved to areas away from where seals haul to avoid disturbance.

Import/Export of Biological Samples (Parts)

Biological samples (hard and soft parts) from Hawaiian monk seals may be exported/re-imported for analysis, per list of cooperating agencies below. Currently the only non-US cooperators are in Canada. HMSRP would export (with subsequent re-import) to any country (world-wide) in the event additional cooperators are identified to run analyses. All exports would be from Honolulu, Hawaii, a U.S. designated port. Samples of Mediterranean monk seals (*Monachus monachus*) may be imported/re-exported if necessary for analysis related to the conservation of monk seals. CITES permits would be obtained as necessary for all exports/imports.

Supplemental Feeding

Supplemental Feeding Methods: Seals receiving supplemental feeding following temporary captive care under separate permit would be provided with Individually Quick Frozen (IQF) herring in quantities of up to 5% of body weight as frequently as once per day for as long one year. The training to take food from people in captivity would be bridged to a wild context, such that released seals could be gradually “weaned” from human support rather than making an abrupt transition. This technique has not been tried with monk seals to date, although in the past seals have been fed reef fish and IQF herring in shoreline pens during enhancement activities including post-captive care and rehabilitation. Much would depend on the seals’ behavior, as they would need to make themselves available to be fed. In order to "wean" the animal while

keeping it in good body shape, feeding may be more regularly (daily) and involve higher rations at the start of the supplementation, then gradually reduce.

Supplemental Feeding Mitigation: Supplemented seals would be pre-trained to approach on cue for feeding, thus non-target seals would very likely not try to obtain provisions. Any uneaten portion of herring offered to a seal would be collected and disposed of properly to keep any waste out of the natural environment. Supplemental feeding of wild seals would occur only in the NWHI where human presence is minimal. It would not be conducted in the MHI, to avoid the problem of these seals approaching members of the public as a food source.

Behavioral Modification

Methods for aversive conditioning

Research is proposed to reduce interactions between humans and monk seals that are potentially or actually harmful to either species. Up to 20 seals may be taken annually in the wild during research and development of aversive conditioning techniques, and 20 annually thereafter for enhancement. Aversive conditioning techniques may be tested on seals in captivity prior to testing on wild seals when appropriate (see Table 2 of Appendix B).

Techniques may involve aversive conditioning, where seals behaving in an undesirable fashion are exposed to unpleasant (but not harmful) experiences in order to discourage the undesired behavior. A variety of aversive and disruptive stimuli may be considered for behavioral modification. While the specific stimuli would be varied they would fall under the following general categories:

- Visual and aural disruptive stimuli: These are intended to stop a seal from its current behavior. This could involve various types of stimuli (shouting, noise making, waving large objects near seals) that disrupts a behavior or displaces a seal from an area.
- Tactile harassment: This includes any technique that repels seals or stops a behavior by direct contact, including prodding with blunt objects (e.g., poles), crowding boards, or low-velocity objects tossed or projected, etc.
- Acoustic Harassment and Deterrents: designed to cause temporary annoyance, discomfort or to frighten seals to displace them from specific locations where conflict occurs. This could include seal crackers (similar to a small firework), underwater speakers, etc.
- Chemical: This includes any chemical that may be used to alter the taste of prey seals obtain in an undesirable ways (e.g., by depredating fishers' catch, bait or gear) or is used to cause temporary minor discomfort to seals to displace them from an area or stop particular behaviors.

In addition to aversive stimuli, positive reinforcers may also be researched and developed to replace the reinforcement of interacting with humans. Tools and techniques would be developed in a careful experimental fashion, and if proven safe and effective, applied as appropriate as an enhancement tool.

Tests of visual, acoustic and tactile aversive techniques

Trials will be conducted to test the effectiveness of visual, acoustic and tactile stimuli to modify behavior of seals interacting with people. Seals would be excluded from aversive testing if they are pre-weaned, compromised by significant injury or emaciation, or if they are pregnant,

lactating or molting. The selected stimulus will be applied multiple (typically three) times to each individual seal to evaluate consistency in induced responses and any habituation. Testing may occur on land or in the water.

Each trial will involve first observing and recording the behavior of the seal without any stimulus. Next, the seal will be approached its response to human approach recorded without application of any aversive stimuli. If the seal flees the area, this indicates that the seal is currently behaving in a manner that is not of concern and the trial would be aborted. Otherwise the trial will continue with a second human approach with the application of the aversive tool. The technique will be repeated or continuously applied, if applicable, until the seal departs the immediate area or until 15 minutes has passed, whichever occurs first. The seal's response will be recorded. Follow up monitoring of the general area will be conducted to determine if the study subject returns to the area or relocates.

Analysis and evaluation
Statistical analyses will be conducted to assess categorized responses of seals to: no stimulus, simple approach without aversive techniques, and aversive stimuli. Sex, age, habitat (land or water), category of undesirable behavior and the trial sequence (first, second, third, etc.) will be examined as covariates. Aversive techniques demonstrated to be effective in modifying undesirable behavior over time will continue to be applied as needed and seal responses will be recorded. Multiple trials following this standardized design may be conducted within the constraints specified in the take table in order to evaluate novel or refined aversive tools.

Tests of chemical stimuli on captive monk seals
Taste aversion safety and efficacy will initially be conducted only on captive seals, so that effects can be documented and any medical support needed can be provided. Seals brought into captivity for two distinct reasons may be tested. First, free-ranging seals that habitually take food from people may be brought into captivity for testing for a duration of 21d. Second, male seals brought into captivity to mitigate their aggressive behavior may also be tested for taste aversion.

The captive seal will be offered a food type that is not part of its current typical diet until it has been established that the seal will willfully ingest this food type. Thereafter, the food will be prepared with a chemical deterrent (lithium chloride) and offered to the seal. The seal will then be monitored and its reaction recorded for at least 12 hr thereafter. Negative physical reactions may include vomiting, inappetence, stomach convulsions, possible ataxia/uncoordination due to muscle stiffness. Supportive care would be provided if seals experience unintended side effects like muscle stiffness and difficulty with locomotion.

If a negative reaction occurs, on the proceeding day the seal will be again offered the food type without the chemical substance, monitored and reaction recorded. If the seal avoids the food type, then it will again be offered to the seal several days later to determine if the avoidance persists. Alternatively, if no avoidance is apparent the food type may be prepared with a higher dose of lithium chloride and the above steps repeated.

Behavioral Modification Mitigation: The purpose of behavioral modification is to mitigate undesirable human-seal interactions, which pose a threat to the seals involved continuing to live

in the wild. Further, these measures are meant to mitigate public safety concerns, which may also affect public attitudes toward conservation of the species.

The behavior modification program would be a joint effort between NMFS and their partners, with the primary lead being the HMSRP initially (during development). This partnership would also have a public nexus as it would require participation by the community in reporting and describing seal behaviors and interactions throughout the process. The HMSRP will establish a Behavior Modification Advisory Committee (BMAC) that will consist of a group of researchers and managers (internal and external) to help with the development and implementation of the program. This committee would also serve to advise on whether an animal of concern is a candidate for behavioral modification trials or application, continue to advise as each case progresses, and provide recommendation for modifying techniques. These techniques would be reviewed and approved by an IACUC prior to implementation.

The program would also consist of implementation teams, which would conduct on-site monitoring and documenting behaviors/interactions and applying any behavioral modification methods. Implementation teams would receive training to maintain consistent data records, safety protocols, and application of behavior modification techniques. It is important that these techniques be administered properly according to a standardized research plan designed to address the specific behaviors displayed by each seal, accurately record the efficacy of methods applied. Therefore only people that have proper authorization and training would be allowed to apply behavioral modification techniques, including aversive conditioning techniques. A core mission of these teams would also be conducting outreach to explain the actions being undertaken and educating the public on proper behaviors to prevent the socialization of seals with humans.

Behavioral modification techniques would be applied only in situations where wild seals are beginning to regularly demonstrate behaviors that put themselves or humans at risk. Again, aversive stimuli that may be used would be tested carefully and while they may cause discomfort or annoyance, would not cause physical harm to seals.

Chemical Behavioral Modification of Adult Males in Permanent Captivity

Chemical Modification Methods: As described above, the HMSRP proposes to mitigate adult male seal aggression by a variety of means. Males identified as aggressors may be translocated, brought into permanent captivity or as a last resort, lethally removed. Each of these methods has drawbacks. All the above approaches can also be logistically complex and quite expensive, factors that also limit their viability. Finally, in cases where the identity of male aggressors is suspected, but not unequivocal, permanent removal efforts (captivity and euthanasia) are not desired. We propose to develop another tool for mitigating male aggression that is effective, humane, feasible, affordable and reversible.

Results of research on chemical alteration of testosterone levels of adult male Hawaiian monk seals indicated that treated males usually responded by exhibiting lower testosterone levels (Atkinson et al, 1986; Atkinson and. Gilmartin, 1992). These studies involved captive and wild seals and included the use of a gonadotropin-releasing hormone (GnRH) agonist (Decapeptyl).

However, the studies did not address whether aggressive behavior was reduced. Other drugs (e.g., Deslorelin) have also been used in a variety of species to reduce testosterone production and aggression.

We propose to conduct research to better elucidate the potential use of a GnRH agonists as a tool for mitigating adult male monk seal aggression (multiple or single males). Research would involve captive trials on Hawaiian monk seals (see Table 2 of Appendix B) or other pinniped species (under separate permit or authorization). Currently there are 4 male monk seals in permanent captivity in facilities in the U.S. Over the five-year period of this permit if additional adult males are removed from the wild into permanent captivity, an estimate of up to 10 adult males in permanent captivity could be taken annually by capture, restraint, sedation, sampling (blood, swabs, blubber biopsy, vibrissae), and administration of a testosterone reduction agent similar to that used previously on monk seals and other similar species. Seals could be captured, sedated, sampled and treated with a GNRH agonist (IM injection or other method deemed safe and effective) up to three times annually. Testosterone would be measured in addition to standard health screening tests. Chemical alteration could also occur via injections by remote administration (e.g., a jab pole). Behavior of treated seals and controls would be monitored prior to and following injections to determine whether levels of aggression are evident following treatment.

If the method proves safe, effective, feasible, and reversible, it could be used as an efficient enhancement alternative to translocation, captivity, or euthanasia of seals in the wild and employed at any location in the Hawaiian Archipelago (an amendment would be requested under the proposed permit prior to use in the wild).

Research and Enhancement—Vaccination

Vaccination Methods: Up to 1,100 monk seals (essentially the entire species) could be vaccinated if the need were to arise and safe, effective vaccines were available to meet that need. The following describes the proposed approach to vaccine studies and vaccination.

Vaccine research
To prepare for and respond to an epidemic caused by morbilliviruses or West Nile virus, the following research is proposed.

Surveillance for morbillivirus and West Nile infections—To enable detection of novel viral infections in the Hawaiian monk seal population, there is a need to routinely and actively monitor for infections. Monitoring wild monk seals for these viruses may include tests for antibodies against the virus in blood (e.g., enzyme linked immunosorbent assays), tests for actual virus in blood, feces, or nasal swabs (e.g., polymerase catalyzed reaction assays), and syndrome-based surveillance. Sample and data collection for these tests would be covered by health assessment studies as described above.

Assess the safety and efficacy of the recombinant CDV vaccine—Currently, one captive Hawaiian monk seal has been vaccinated against morbillivirus. Vaccination of additional Hawaiian monk seals would better elucidate their ability to mount a proper immune response, the

number of vaccines (including boosters) needed to generate this response, and the duration of immunity against morbilliviruses. Vaccination of additional captive Hawaiian monk seals will be pursued with partners under separate permits, including the Waikiki Aquarium and Sea World San Antonio, which are both authorized to conduct this research under their own permits. We request authorization to conduct vaccine research on monk seals in other facilities that do not have permits to conduct the research.

Post-Vaccination Antibody Response (PVAR) Methods for Permanently Captive Monk Seals
Captive seals can serve as a model to establish vaccine antibody response for Canine distemper virus (CDV) and West Nile virus (WNV). For CDV, the use of Purevax (Meriel) would be used (a monovalent recombinant canary pox). Recombinant vaccines pose less risk than use of a live virus. The WNV vaccine is a product made by Fort Dodge of inactivated WNV. As an inactivated virus, it cannot be shed and therefore does not require a closed system. In addition, the recombinant canary pox has been tested in harbor seals at Sea World (by Pam Yochem) and no virus shedding was detected (Dr. Frances Gulland, personal communication).

To assess the effectiveness of the vaccines, serum antibody samples must be taken throughout the year. It is proposed to collect serum on days 0, 28, 42 and 365 to monitor antibody formation. Day 0 serum collection will occur prior to vaccination to provide baseline values for each animal. Vaccination for both CDV and WNV will occur after the serum is collected. Along with serum samples, duplicate nasal swabs will be obtained. A follow up vaccine will be given on day 14, but no blood sample will be taken at this time. Each vaccine is given subcutaneously in a 1 ml dose, administered twice, fourteen days apart. To minimize restraint and handling time of the seals, the serum collections on days 0 and 365 may also serve as annual blood sampling for the seals regular health monitoring. Additional handling and sedation will occur on days 28 and 42 post-vaccination to obtain the serum and nasal swabs only.

For both routine health monitoring and the PVAR study, blood samples will be obtained through the use of chemical sedation if deemed necessary by the attending veterinarian and light physical restraint. Sedation would be achieved with either diazepam (0.2 mg/kg IV) or midazolam (0.2 mg/kg IM) and blood collected from the extradural sinus or interdigital webbing vein. Flumazenil will be kept on hand for emergency use to reverse diazepam or midazolam sedation if necessary. However, it will not be used routinely as the half-life is less than that of the sedative drugs. Blood samples and nasal swabs will be obtained. At some facilities, seals may be trained for voluntary blood sampling. In addition, vaccination of future monk seals brought into temporary captive care for medical care (under the MMHSRP permit) may be conducted during the research phase.

Outbreak response for seals in the wild
Vaccination of monk seals may occur either in response to an outbreak or prophylactically in the absence of disease in Hawaii. Once a minimum of five captive seals has been vaccinated with no adverse effects identified, a prophylactic vaccine trial should be developed in the MHI. However, until this trial has been performed, a response plan is needed in case of disease events that could significantly increase the risk of morbillivirus disease in monk seals, due to their critically endangered status. A series of different disease parameters in Hawaiian monk seals, other marine

mammals and domestic animals have been identified that could trigger a vaccination response in Hawaiian monk seals.

HMSRP proposes to vaccinate in response to disease outbreaks as diagnosed by a series of triggers described below. If the risk of morbillivirus or West Nile virus epidemics to monk seals changes from the current situation, this approach may be modified.

Morbillivirus

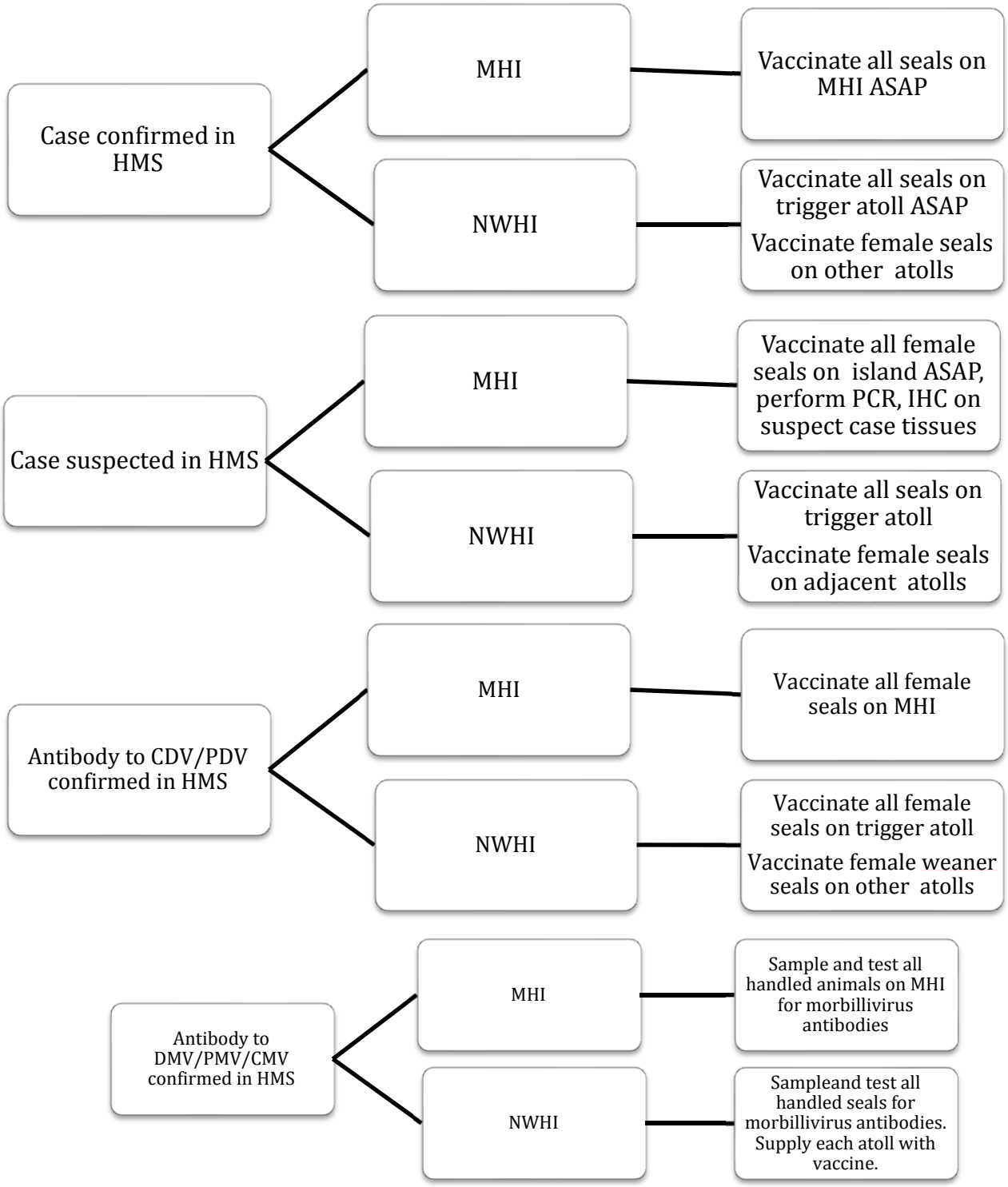
Triggers
A *confirmed* case is an animal with pneumonia, or encephalitis, or lymphadenitis, or dermatitis, with morbillivirus detected in tissues by PCR or immunohistochemistry, and its identity confirmed by nucleic acid sequencing.

A *suspect* case is an animal with severe pneumonia or encephalitis associated with syncitial cells and inclusion bodies detected on histology, with either a positive PCR or immunohistochemistry result.

Detection of antibody occurs when serum neutralization test results are greater than 1:16.

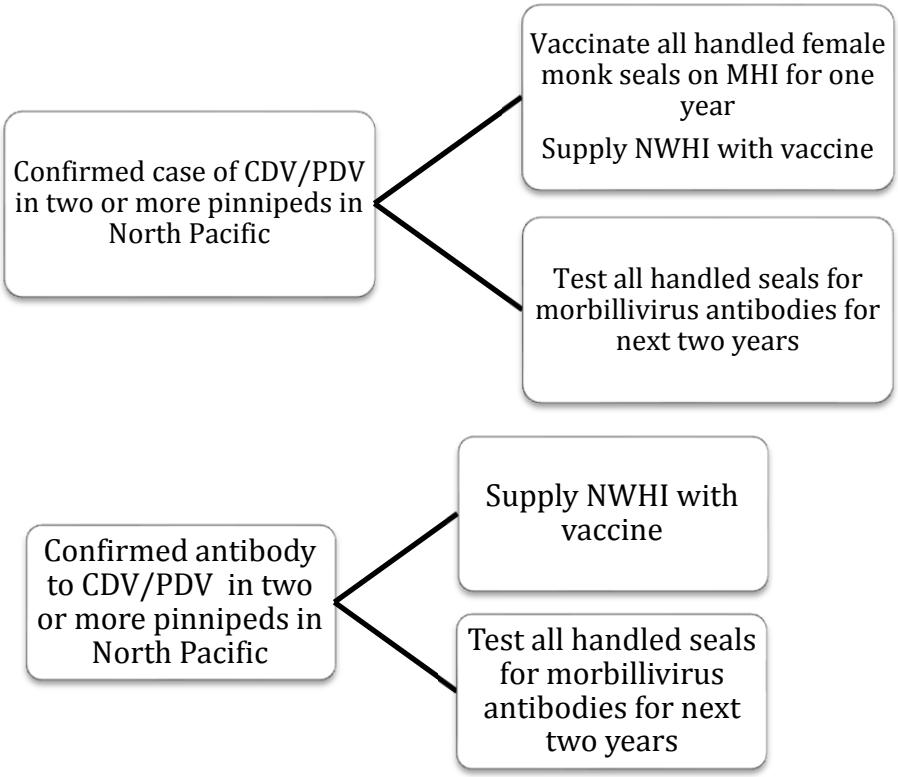
Responses to each disease parameter are summarized in the decision tree below. Each response is made by weighing the advantages and disadvantages, and recognizing that a second trigger occurring during a response may increase the level of response. Detection of antibody implies that exposure is occurring, but lack of disease would imply seals have developed resistance to the exposure. Thus vaccination response would be at a lower level than that to a detected case.
All vaccination responses would be maintained for one year. During response, surveillance for morbillivirus infection through necropsy of dead animals and serology of handled live animals will be prioritized by NMFS. Following vaccination, all vaccinated animals would be blood sampled and tested for morbillivirus antibodies within one year of vaccination unless pregnant.

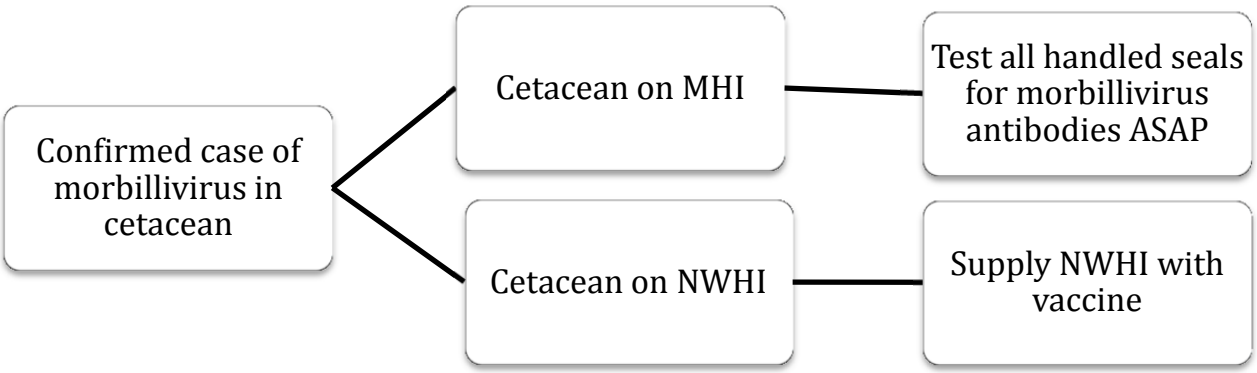
Triggers in Hawaiian Monk Seals

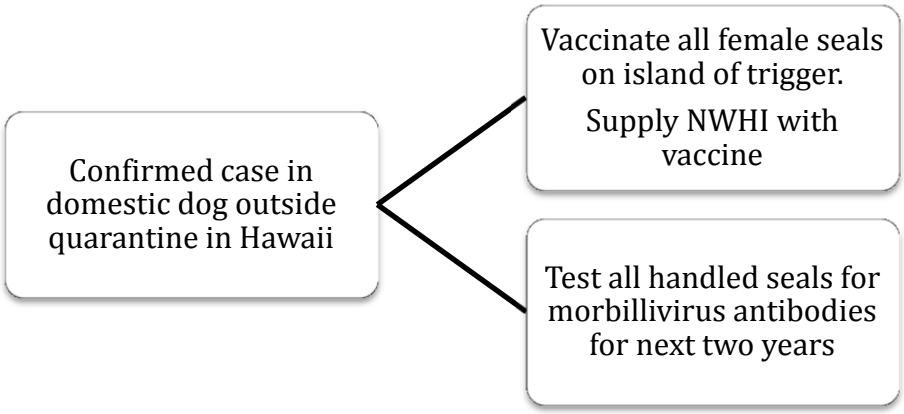


Triggers in Other Mammals

Morbillivirus associated disease in seals to date world-wide is believed to have resulted from transmission of virus from other seal species and domestic dogs (Grachev et al 1989, Jensen et al. 2002). Thus diseases in these species are considered risk factors for monk seals. Morbillivirus disease has not been reported to date in pinnipeds of the North Pacific, nor in mammals on the Hawaiian Islands, despite its prevalence in seals in Europe and the Atlantic (see above), and in domestic dogs in the continental United States. If morbillivirus disease was detected in pinnipeds in the North Pacific, the risk of Hawaiian monk seal exposure to morbillivirus infections would be heightened due to occasional movement of pinnipeds from California to Hawaii. Movement of pinnipeds from the mainland United States to Hawaii occurs unpredictably, and vaccination takes time to perform and achieve protective immunity. Thus, triggers that suggest pinniped morbillivirus disease could reach Hawaii at random times have been identified to trigger vaccination. Triggers that could occur in mammals other than pinnipeds have also been identified.







Results of the response to the first trigger event will be used to refine responses to subsequent trigger events. In particular, records will be taken on:

- Time between trigger and administration of first and second dose of vaccine;
- Number of seals vaccinated;
- Time required to vaccinate all or most animals on island;
- Age distribution of vaccinated animals;
- Resightings of vaccinated animals; and
- Any indication of adverse reaction to vaccination.

West Nile Virus

The epidemiology of West Nile Virus differs significantly from that of morbilliviruses, as it is a vector borne zoonotic virus rather than a directly spread animal pathogen. This virus caused the death of a captive monk seal at SeaWorld San Antonio, Texas, and has caused mortality in captive harbor seals in the mainland U.S. To date this virus has not been identified in wild marine mammals, although it is present along the eastern seaboard and southern California. As this mosquito-borne virus is currently not present within Hawaii, the State has rigorous surveillance and response plans for controlling this virus due to its public health importance. Although neither single cases of disease nor epidemics of West Nile Virus have been reported in wild marine mammals to date, the death of a monk seal in Texas from this infection indicates

monk seals are susceptible. Thus, the possibility of extensive mortality in monk seals exists if the virus were to be introduced to Hawaii, warranting a response plan to such a scenario

Trigger
A case of West Nile virus in the Hawaiian Archipelago in humans or wildlife, with activation of the State emergency response for West Nile virus control could trigger implementation of West Nile virus vaccinations in wild Hawaiian monk seals.

Response
As vaccination of Hawaiian monk seals to WNV has occurred with proven safety for over 5 years in 8 captive monk seals in Texas, the risk of vaccination against WNV is minimal, apart from risks associated with approach and injection.

In response to a detected case of WNV in any species in Hawaii, all accessible seals on the MHI would be vaccinated with West Nile virus vaccine (Innovator, Fort Dodge), starting with the island on which the case was identified. Vaccine would be transported to each NWHI and used if the outbreak is not controlled in the MHI within 2 months.

Potential prophylactic vaccination
The best way to protect Hawaiian monk seals against these viral infections is to vaccinate prior to population-wide exposures. This is especially true if multiple doses of vaccines are required to gain immunity against infections, or if immunity responses take weeks to months to develop. Conversely, vaccines that mount short-term responses against infections or have higher risks of side effects may best be delivered only in the face of population-wide exposures. Based upon the information gained from research and any outbreak response, it will be determined whether prophylactic or solely response-driven vaccinations against morbillivirus and West nile virus are needed.

Vaccination Mitigation: The preceding section describes a disease mitigation effort through the potential use of vaccination. Risks specifically associated with vaccination (separate from disease risk) would be mitigated by the cautious, stepwise approach to determining safety and efficacy of specific vaccines and applying them judiciously when the threats of disease warrant their use. Implementation of any vaccination program would be properly scaled to particular triggers as described above.

Incidental Harassment
Up to 400 seals of any age/sex may be incidentally disturbed while researchers conduct research and enhancement activities on other seals and during opportunistic sample collection (e.g., scat, molt). Hawaiian monk seals hauled out on the beach may also react to human presence at field camps (i.e., living in tents), small boat operations (transits and landings to support the work and set up camps), and installation and maintenance of remote video cameras.

Measures to Minimize Harassment: Observers remain as far away as possible from seals during monitoring activities to obtain the necessary data, using binoculars and telephoto lenses as necessary for documentation. All field staff are trained to be unobtrusive and to remain low to the ground whenever seals may alert to human presence. Seals are specifically given a wide

berth when they are especially susceptible to disturbance, such as lactating females or molting individuals.

Additional Information for Captive or Rehabilitating Animals

a. Explain why removal from the wild is necessary and why suitable animals cannot be obtained from captive or rehabilitated stock.

> Non-releasable captive seals used for scientific research purposes would already be in captivity.
>
> For enhancement, removing adult male seals would occur to prevent severe injury and death to conspecifics. Also, weaned pups and juveniles may be held captive during quarantine as part of the two-stage translocation enhancement project.

b. Provide a description of the enclosure or cage to be used for temporary holding and transport, mode of transportation, name of transportation company, special care during transport, and the length of time required for the transfer from the capture site to the initial holding facility, and then to the permanent holding facility. Provide a list of personnel who will be involved in and/or accompanying the animals during transport and whether a veterinarian will be present, and their qualifications.

> Subject seals already in captivity will not be moved between facilities on this permit unless specifically requested. If requested, specific transport information will be provided for approval.
>
> Seals removed from the wild for enhancement purposes will be transported by ship or plane and truck. Transport cages (e.g., 8 ft long x 4 ft wide x 4 ft high) may be used for transport and very short term holding (e.g., up to one week in cage for transport and holding). Smaller cages may be used for small seals. Seals would be able to lie flat and turn around in cages.
>
> Transport times would vary depending on the location of capture. Specific transport plans would be documented in accordance with APHIS requirements for transports greater than 2 hours and would include the personnel accompanying the animals, whether a veterinarian is deemed necessary for the transport, the health status of the seals, monitoring and care during transport, duration and mode of transport.
>
> If during transport a seal becomes sick or injured, veterinary and husbandry staff would be on board and would have a full kit of emergency drugs, antibiotics, intubation equipment, fluids for hydration, and equipment and supplies to provide nutritional supplementation to weaned pups (e.g., herring slurry, electrolyte fluids) or adults (e.g., IQF herring) if necessary. The compromised seal(s) will be kept on board and taken to Honolulu. During transit the seal(s) will be monitored 24 hours/day and treated appropriately by the attending veterinarian. If captive

care for medical reasons is warranted, seals would be cared for under the MMHSRP permit. Releasability of seals intended for translocation will be discussed with OPR on a case-by-case basis. Adult males would remain in captivity.

c. Rehabilitating marine mammals: If the source stock is to be beached/stranded marine mammals undergoing rehabilitation, indicate the name and location of the rehabilitation facility.

Seals undergoing rehabilitation in Hawaii may be used in the vaccination research. Rehabilitation facilities may include the Ford Island NOAA facility, the Waikiki Aquarium, and The Marine Mammal Center will be building a facility on the Big Island of Hawaii.

d. If the source stock is from animals already in captivity (other than beached/stranded animals in rehabilitation) indicate the name and location of the facility and identify the specific animals (by NOAA ID number where applicable) involved in the proposed activity.

Permanently captive Hawaiian monk seals include the following:

Currently at Long Marine Lab:

Hawaiian monk seals held at Long Marine Laboratory, Santa Cruz, CA, for scientific research (Permit No. 13602-01).			
Seal ID/Name	NOAA ID	Estimated date of birth	Sex
KE18	NOA0006781	May 1, 2002	Male
Up to 2 additional non-releasable monk seals may be held at any given time (age, sex, and ID's to be determined).			

Currently at Sea Life Park:

Hawaiian monk seals held at Sea Life Park, Waimanalo, Hawaii, for enhancement purposes under Permit No. 898-1764-01.			
Seal ID/Name	NOAA ID	Estimated date of birth	Sex
T711, "Spruce"	NOA0005666	May 1, 1980	Male
YL79, "Lambchop"	NOA0005662	May 20, 1986	Female
Two additional seals may be held at Sea Life Park.			

Currently at Waikiki Aquarium:

Hawaiian monk seals held at the Waikiki Aquarium, Honolulu, Hawaii, for scientific research and enhancement purposes under Permit No. 455-1760-01.			
Seal ID/Name	NOAA ID	Estimated date of birth	Sex
Y294, "Maka"	NOA0005663	April 15, 1984	Male
KP2, "Ho'ailona"	NOA0006753	May 1, 2008	Male
Up to one additional seal may be held at the Waikiki Aquarium.			

Currently at Sea World San Antonio:

Hawaiian monk seals held at Sea World San Antonio, Texas, for enhancement purposes under Permit No. 116-1786-03.				
Seal ID/Name	NOAA ID	Sea World ID	Estimated date of birth	Sex
YC14, "Ola"	NOA0005646	SWT-MS-9978	May 1, 1995	Female
YC16, "Paki"	NOA0005651	SWT-MS-9979	May 1, 1995	Female
YC28, "Nani"	NOA0005653	SWT-MS-9983	June 19, 1995	Female
YC32, "Koa"	NOA0005656	SWT-MS-9984	June 15, 1995	Female
YC35, "Opua"	NOA0005654	SWT-MS-9985	June 15, 1995	Female

If other monk seals are deemed non-releasable to the wild and held at these or other facilities, we request permission to conduct these studies on additional seals. Their ID numbers and facility information will be provided if this occurs. Location of research could be at any facility listed in this application as well as any other facility approved by APHIS and the NMFS Permits Division to hold Hawaiian monk seals.

e. For marine mammals: Include a copy of any license or registration issued by the Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture, any outstanding variances granted, and the most recent APHIS inspection report.

For scientific research on permanently captive, non-releasable seals already in captivity, the seals would not be maintained in the facilities under this permit; only the proposed research would occur there. Each facility is responsible for providing these documents to the Permits Division as part of their requirements to hold monk seals in captivity.

For enhancement, adult male seals could be authorized under this permit temporarily at the Ford Island Facility, The Marine Mammal Center Facility, or an APHIS-approved facility until such time that they can be transferred to a permanent home if other facilities have agreed to take the male(s). If another facility is willing to take an adult male and does not currently have a permit, we request permission to maintain the seals at other facilities approved by APHIS until such time as the facilities are able to obtain their own permit. Prior to such arrangements, the HMSRP would seek approval from the Permits Division and provide the necessary APHIS documentation.

Also for enhancement, translocated seals that are part of the two-part translocation program may be held in temporary quarantine in the above-mentioned facilities.

f. Provide a written statement from the responsible veterinarian or expert certifying that the facilities, methods of care and maintenance, and methods of transport will be adequate to ensure the well-being of the animals and, for marine mammals, will comply with all care and transport standards established under the Animal Welfare Act (AWA).

For scientific research, the seals would not be maintained under this permit; only the research proposed would occur. Each facility is separately permitted/licensed under the AWA and must meet the regulatory requirements under the AWA to hold monk seals. The research would be reviewed and approved by an IACUC.

For adult male removal and two-stage translocations (enhancement), such statement would be provided prior to removing the seals from the wild into permanent captivity.

g. For ESA-listed species: Describe the care and maintenance of the animals, including a complete description of the facilities where they will be maintained. This includes the dimensions of the pools or other holding facilities and the number, sex, and age of animals by species to be held in each; the water supply, amount, and quality; the diet, amount and type; sanitation practices; and qualifications and experience of the husbandry staff.

The proposed research with permanently captive Hawaiian monk seals will take place at facilities that have been previously approved by NMFS and APHIS for their holding: Sea World- San Antonio, Sea Life Park (Oahu, HI), Waikiki Aquarium (Oahu, HI), Long Marine Laboratory (Santa Cruz, CA) and any other facility approved by APHIS and NMFS to hold monk seals. Details of the facilities and husbandry protocols can be provided upon approval of a collaborative agreement if necessary, but each facility must provide that information to the Permits Division to obtain the permits to hold the seals.

During translocation projects, it will sometimes be necessary to temporarily hold seals captive on the beach (especially in the NWHI). For example, when collecting seals from a given subpopulation, the subjects may need to be gathered together over the course of several days so that they can subsequently be efficiently and safely transported to a ship or plane. Likewise, seals may be held at their destination for some time prior to release. The primary structure for temporary holding (longer than approximately two days) will be shoreline pens, measuring up to approximately 24 ft x 80 ft. Approximately 30 percent (%) of the surface area will include water at least 2 ft deep at lowest tide. The remainder of the pen would be intertidal and dry resting area above the high water line. No more than 5 seals would be held in a pen at any one time. In some instances requiring short temporary captivity (e.g., less than two days), a shaded holding pen may be erected in the vicinity of the field station, and seals would be wetted down periodically.

Pens will be constructed from plastic or metal (typically mesh) material, approximately 4 ft high, supported by approximately 10 ft x 2-3 in diameter steel pipe driven into the sand at approximately 8 -10 ft intervals. Pipe or water filled fire hose will be used to secure the bottom of the fencing material. Plastic ties will fix the fencing to the support piping and bottom weights, and windbreaks will be erected along the fence as necessary. Fence perimeters (in and out of water) will

be monitored at least twice daily, and will be repaired or changed as necessary to prevent escape or injurious entrapment. Alternate but comparable construction materials or pen configurations may be used within the range of dimensions described above.

Also for enhancement (male removal and translocation quarantine), seals could be temporarily maintained under this permit at the Waikiki Aquarium, the planned Ford Island Facility on Oahu, or the planned Marine Mammal Center facility on Hawaii Island. There are two pools at the Waikiki Aquairum 20 ft in diameter and 4 ft deep with floating haul outs. At Ford Island, there will be four pools 20 ft in diameter 4 ft deep. Available deck space will be 361 square feet (sf) for each pool. The current plan for the Marine Mammal Center facility includes four pools, two larger and two smaller. Larger pools would be 20 ft by 12 ft and 5 ft deep, with approximately 830 sf of haulout decking. The small pools would be 6 ft by 6 ft and 3.5 ft deep, with approximately 250 sf of haulout space. If additional facilities become available (e.g., to hold adult males), those facilities' specifications will be provided.

For temporary captivity of adult males (removed from wild) under this permit, an experienced marine mammal veterinarian will be present throughout acclimation and temporary holding. At least one NMFS staff experienced with monk seal biology and behavior will help monitor and care for the seal. Trained volunteers may be used for monitoring and cleaning. Seals will be monitored and disease screened using standard protocols (Appendix F). Seals will be offered IQF herring daily and fed up to 5% of estimated mass per day as determined from appetite, behavior and defecation. Food preparation, feeding, and cleaning will follow established NMFS/TMMC protocols. The attending veterinarian will monitor the seal on a daily basis during acclimation. In the event the seal exhibits signs of distress or illness, the attending veterinarian would respond using protocols for emergency response, and would use veterinary discretion for treatments including euthanasia for medical reasons.

h. Indicate whether a captive breeding program will be established and if so, provide justification in accordance with the species conservation or recovery plan as applicable for enhancement activities. For ESA-listed species, indicate if you are willing to participate in a captive breeding program if requested by NMFS.

N/A, no captive breeding involved in this study.

i. Indicate the disposition of captive animals at the termination of research or enhancement activities.

Animals in permanent captivity will remain in their original facility at the termination of this research. Any adult male removed for enhancement purposes would remain in permanent captivity.

j. If release of captive animals to the wild is proposed.

N/A, release to the wild of permanently captive animals is not proposed. Seals undergoing rehabilitation and used in vaccination research would be rehabilitated and released under separate authority and following NMFS rehabilitation guidelines.

Project Supplemental Information

Status of affected species

Hawaiian monk seal: The Hawaiian monk seal is listed as endangered under the ESA and depleted under the MMPA, and is listed on CITES Appendix I. The status of the Hawaiian monk seal has most recently been described by Caretta et al. (2009), which presents information through 2007. To summarize, The best estimate of the total population size was 1,146 and abundance at the six main NWHI subpopulations was estimated to be declining by 4.1% per year. Actual trend information is not available for the relatively small MHI subpopulation, but a simulation model projection using vital rates estimates for seals in that region suggests the MHI seal population may be growing at approximately 7% per year.

Non-target species: The Pacific population of the green turtle is ESA threatened, and IUCN endangered worldwide (no IUCN listing for separate Pacific population).

The Laysan finch, Laysan duck, Nihoa Miller bird, and Short-tail albatross are ESA endangered and IUCN vulnerable.

The spinner dolphin is CITES Appendix II and IUCN lower risk. Spinner dolphins to be taken are part of the Hawaiian stock, subspecies *S. longirostris longirostris*. The bottlenose dolphin that may be affected is from the Hawaiian Islands Stock Complex. The bottlenose dolphin is CITES Appendix II and IUCN least concern. They are not depleted, threatened, or endangered.

Lethal take

Intentional lethal take of monk seals is described in the project description/methods section of the application and could involve:

- Aggressive males seals lethally removed as a last resort and would be done within the strictures described in the project description section (up to 10 seals over the 5-year period of the permit)
- Moribund seals humanely euthanized or which may die during health screening also described in the project description (up to 10 seals over the 5-year period of the permit)

Unintentional Mortality

Despite all mitigation employed, unintentional mortalities may occur in association with both research and enhancement activities. Intentional lethal removal of adult males and euthanasia of moribund seals was addressed in preceding sections. Below is a characterization and rationale for requested numbers of *unintentional* mortalities.

Unintentional mortality due to research—A total of 4 seals may be taken in 5 years, with no more than 2 seals taken per year. These seals can be of any size and of either sex. Research-related mortalities have been rare. During the past 4 complete years of permitted research, there has been 1 unintentional mortality, for an average of 0.25 per year. Here it is requested that there may be 4 mortalities in 5 years (an average of 0.8 per year). However, mortalities occur in whole numbers only, not fractions, and the total proposed allowed takes (4) is already a small whole number. Thus, while it is unlikely that this level of takes will occur, it is certainly within the realm of reason that 4 lethal accidents could occur over a 5-year period of research. Moreover, this application involves increased research takes in various categories compared to recent years. Many of these takes entail capture, restraint and sometimes sedation, which are the types of activities that present higher risk of unintentional mortality.

Unintentional mortality during enhancement activities—This lethal take is subdivided into three groups:

1) Weaned pup (either sex): 4 pups over 5 years, with no more than 2 in one year
2) Juveniles (either sex): 8 seals over 5 years, with no more than 4 in one year
3) Adult Males: 4 males over 5 years, with no more than 2 in one year.

This permit application entails a dramatic increase in enhancement efforts in comparison to recent years' levels. New or expanded enhancement activities, which might result in increased takes include:

- Weaned Pups
    - Increased deworming
    - Increased translocation for risk alleviation
    - First stage of two-stage translocation
    - Behavioral modification
    - Vaccination
- Juveniles
    - Increased deworming
    - Second stage of two-stage translocation
    - Behavioral modification
    - Vaccination
- Adult males
    - Increased potential number of removals in response to increased multiple male aggression.
    - Initiation of chemical behavior modification

Compounding the risk of simply increasing the number of animals involved in enhancement is that for some of the proposed activities, the inherent risks are not well known. Whereas a large number of weaned pup translocations have been conducted and the level of risk involved is quite low (Baker et al. 2011), far fewer cases of juvenile translocations have occurred. The general sense, however, is that juvenile seals are at greater risk of stress and mortality when being held captive. In a 2006 captive care program at Midway Atoll, 6 weaned pups and 1 juvenile seal were held in shoreline pens to be fattened up. All the pups gained weight and were released in

good body condition, while the single juvenile died of complications related to stress a few weeks after being brought into captivity (Baker and Littnan, 2008). Because juveniles seem subject to greater risk in captivity, the number of requested lethal juvenile takes is higher than that for weaned pups (4 in 5 years), notwithstanding the fact that more weaned pups are likely to be involved in enhancement activities. Compared to translocation, other enhancement activities with young seals (deworming, behavioral modification, vaccination) are thought to present lower risk. However, these are either entirely new or only rarely tested activities, so that their true risks remain uncertain and difficult to quantify pending initial trials.

A final risk magnifier that is reflected in the number of proposed unintentional mortalities is that some activities, most notably two-stage translocations, involve "grouped risk" whereby several animals will be captured, transported, held in quarantine and released together. In statistical language, by grouping seals in this way, the risk of unintentional mortality becomes "non-independent". That is, if some rare but lethal event should occur (disease outbreak, boating or vehicle accident, etc.), there is greater likelihood of losing multiple seals at one time.

It is expected that the benefits of the proposed enhancement activities will indeed greatly exceed the potential losses due to unintentional mortality.

Other anticipated effects on animals

Individual animals may be affected by presence of observers on the beach during ground counts/monitoring activities if they alert to the observer and flee into the water. Most seals, however, even upon seeing the observer, do not flee, but rather alert, raise their head, or perhaps vocalize. Levels of disturbance are noted during field observations, and are coded according to whether the seal: does not notice the observer (0); alerts and/or moves less than two body lengths (1); moves two or more body lengths but remains on the beach (2); or flees into the water (3). Seals are not expected to react to the presence of aircraft surveying from altitude. Based upon observations during aerial surveys, seals very rarely react to aircraft and when they do, typically just raise their heads momentarily. Observations of seal responses to non-survey aircraft in the MHI are consistent with the conclusion that they are typically not bothered by even large, low-flying aircraft.

Individual seals may awaken during marking, with and without seeing the person applying the mark. Reaction behaviors could include: looking around, vocalizing at observer, moving short distance away, or fleeing into the water. Short term effects are brief expenditures of energy. No long term effects are anticipated.

Individual seals will experience some varying degrees of stress and discomfort when being restrained, depending upon the activity undertaken. Routine tagging will cause temporary stress and some transient pain, and collection of samples for health screening, vaccination and de-worming may cause additional levels of stress and some added discomfort. Disentanglement from marine debris will also cause temporary stress, but will probably save the animal from death or serious injury. Attachment of instruments will also increase stress, because the individual seals will be held for longer durations. All animals restrained for health screening or instrumentation will be sedated under the direction of an attending veterinarian as necessary to

reduce stress. There is some risk to individual animals of mortality during restraint, especially moribund animals.

Animals which are translocated will experience stress during capture, handling, short-term captivity, and transport. Abandoned nursing pups which are relocated to a potential foster mother will in particular experience stress and will risk injury due to possible aggression by a non-receptive female. However, abandoned pups are certain to die if they do not suckle, so the potential benefit of being able to suckle far outweighs any injury the animal may receive soliciting milk from a non-receptive female. The anticipated effect of the activity on individual animals is increased chances of survival, and may contribute to increased chance of species recovery. However, Baker et al. 2011 have analyzed past translocation of Hawaiian monk seals and found that translocated seals survived just as well at their release sites as native (non-translocated) seals at the same sites.

The HMSRP has conducted three studies to assess whether research handling has any adverse affect on the Hawaiian monk seal population. Henderson and Johanos (1988) determined that capture, restraint, and flipper tagging had no effect on subsequent behavior of weaned pups. Baker and Johanos (2002) conducted an extensive study, examining the effects of tagging, blood sampling, and instrumentation with telemetry devices. There were no effects of tagging/handling on survival, migration, or condition of the tagged seals. Moreover, sample sizes were sufficient to detect a 9% difference in resightings. The authors conclude that conservative selection procedures and careful handling techniques have no deleterious effects on Hawaiian monk seals. Littnan et al. (2005) measured the impact of Crittercam attachment on the dive behavior of juvenile monk seals. They found that for short duration deployments (< 2 weeks) there was no detectable difference in dive behaviors (i.e., dive depth, dive duration, foraging trip duration) with or without the camera attached. Crittercams are the largest and least hydrodynamic of the telemetry instruments being deployed so it is unlikely other technologies are impeding the seals abilities to forage.

Anticipated effects on the population as a whole

Potential population- and species-level effects of the proposed activities have been thoroughly analyzed in the *Draft Hawaiian Monk Seal Recovery Actions Programmatic EIS* (Chapter 4), hereafter referred to as the "PEIS". The following is a summary of those analyses and the conclusions for the preferred alternative, which is the basis for this permit application.

The PEIS analysis looks at three types of impacts on Hawaiian monk seals:

1) Effects on mortality
2) Effects on reproductive success
3) Beneficial contributions of actions to conservation of the species.

Analysis of mortality effects associated with research and enhancement activities were primarily based on up to three sources of lethal takes described previously. These include:

- Adult male removals. These involve either lethal removal or permanent captivity of adult male seals that have harmed or killed other seals. Because permanent captivity is equivalent to mortality from the perspective of the wild populations, captivity was treated as a mortality in the analysis.

- Unintentional mortality (research). This includes any unintentional deaths of seals that may occur as a result of research.
- Unintentional mortality (enhancement). This includes any unintentional deaths of seals that may occur as a result of enhancement activities.

These sources of mortality are considered to be entirely observable. The HMSRP has a long history of evaluating the potential effects of research and enhancement on Hawaiian monk seals as evidenced by numerous published reports and papers showing that Hawaiian monk seals subjected to specific research and enhancement activities do not subsequently exhibit higher mortality than seals not subjected to the activities (Baker and Johanos 2002; Littnan et al. 2004; Baker et al. 2011). Moreover, these studies have often sought to detect sub-lethal effects (for example, on behavior, movement, body condition, etc.) of research and enhancement activities, but have failed to find evidence of any such deleterious effects. Based on these publications, coupled with the fact that most Hawaiian monk seals are uniquely identifiable and closely monitored, it is assumed that there are no unobserved mortalities associated with research and enhancement activities.

Thus, the HMSRP concludes that the unintentional or intentional (in the case of aggressive adult male seals) mortalities that are observed as an immediate result of research or enhancement constitute the totality of mortality associated with these activities under this permit. It is important to note that this is not a claim that research and enhancement have no associated mortality; rather it asserts that such mortality will be entirely observable and documentable.

Regarding reproductive effects, the HMSRP has a long-standing conservative approach to disturbance or capture of reproductive female Hawaiian monk seals. For example, no adult female is captured that appears to be pregnant or is otherwise thought likely to be well into a pregnancy even if it is not visually apparent. The only exception is for a life-threatening situation such as a severe entanglement. Also, great efforts are made to minimize the disturbance of mother-pup pairs. Because of these precautionary policies, the risks to reproductive females are minimized, but at the same time risk-averse procedures complicate any analysis to evaluate whether any effects are occurring. For example, in the Baker and Johanos (2002) study on effects of research handling, reproductive effects could not be evaluated. Because pregnant females were actively avoided in the study, there were no control seals to compare subsequent reproduction of the adult females that were handled (i.e., the adult female treatment group was biased).

Despite the complications with quantitative evaluation of reproductive effects based on actual research and enhancement activities in the past, it is possible to qualitatively infer the likelihood of such effects. For example, many of the hypothetical mechanisms for reproductive effects are mediated through reduced growth or body condition of female seals. Avoiding handling pregnant females reduces this risk. Also, the lack of any indication that actions such as tagging, health screening, instrumentation, and de-worming have had any negative effects on growth or body condition (Baker and Johanos 2002; Gobush et al. in prep.), suggests that growth-related effects on reproduction are highly unlikely. Likewise, the strict avoidance of disturbance to mother-pup pairs and the prohibition on capturing either a mother or her offspring during the period between birth and weaning, means that effects on the nursing process are also very unlikely. There has

never been a reported or documented case where research or enhancement related disturbance has caused a female to abandon a pup.

It is difficult to evaluate the remaining mechanisms: stress-related reproductive failure or damage to reproductive organs. Again, by avoiding handling pregnant female seals (or those who could be pregnant) the potential for stress-related effects is minimized. Goebel et al. (2003) evaluated the birth rates of female Antarctic fur seals the year following capture, restraint, anesthesia, and post-canine tooth extraction (for age determination) to a control group of females that was not captured. There were no differences detected in birth rates of these two groups. The procedures these fur seals were subjected to were arguably far more intense than any procedure proposed for Hawaiian monk seals. While one cannot assume that results from another species are applicable to Hawaiian monk seals, this information is encouraging. We cannot rule out that handling could damage reproductive organs. On the other hand, if organ damage of any kind did occur, one would expect vital organs important to survival would be as likely, or more likely, to be involved than specific reproductive organs. The lack of any detectable effects on survival described in the preceding sections suggests that vital organ damage, and by inference, reproductive organ damage, is unlikely.

In summary, directly evaluating reproductive effects is far more complex than is the case for effects on survival. While we cannot rule out the potential for reproductive effects of proposed research and enhancement activities, several lines of evidence, including years of monitoring data for Hawaiian monk seals, suggest that this is a minor concern for Hawaiian monk seals.

Beneficial contributions to conservation of Hawaiian monk seals that would be achieved by the proposed research and enhancement activities were both qualitatively and quantitatively analyzed in the PEIS. These positive effects counter any negative mortality (or reproductive) impacts. Some beneficial contributions (such as from two-stage translocation) were quantitatively assessed in the context of a stochastic simulation model that evaluated projected changes in various population metrics given a range of likely translocation scenarios. Other enhancement activities (such as disentanglement) were qualitatively analyzed.

For the activities proposed in this application, the PEIS concluded that the expected small changes in the population (due to mortalities) would likely amount to an equivocal change in population status, so that the magnitude and intensity of mortality effects would be moderate. Further, because the losses amount to a small number of individuals, the geographic extent/biological level of the impacts would be minor. The allowable lethal takes are moderate frequency (no more than a few per year would be likely) and would occur with moderate duration (according to the 5-year permit cycle), such that the duration and frequency would be moderate. The majority of the potential lethal takes of female seals are associated with enhancement activities. These activities will focus on seals that are already at elevated risk of natural mortality and enhancement activities are expected to achieve benefits in improved survival (presented below) The overall adverse direct and indirect effects of research and enhancement on mortality would be minor to moderate adverse. The PEIS also concluded that reproductive effects would be negligible.

The activities proposed were designed to address a majority of the objectives of the Recovery Plan. The PEIS concluded that these activities, especially the ambitious enhancement activities, would likely be a *major* beneficial contribution towards conservation objectives. The balance of all effects (mortality, reproductive and conservations benefits) in total are likely to have a net positive effect on the recovery and conservation of Hawaiian monk seals.

Measures to minimize negative effects

Observers remain as far away as possible from seals during monitoring activities to obtain the necessary data, using binoculars and telephoto lenses as necessary for documentation. All field staff are trained to be unobtrusive and to remain low to the ground whenever seals may alert to human presence. Seals are specifically given a wide berth when they are especially susceptible to disturbance, such as lactating females or molting individuals.

Only seals that are asleep will be bleached. Moreover, molting seals, which are more restless and subject to disturbance, will be avoided. Seals will not be bleach marked if they are sleeping in close proximity to other sleeping seals or basking green turtles, or if extrinsic factors exist that could threaten their welfare if they are startled (e.g. rocks). Seals swimming in the nearshore area sometimes approach another seal that is in turn being marked. In such instances, the swimming seal may notice the biologist and vocalize, alerting nearby animals. We will minimize effects on non-targeted animals by not marking seals when other seals or green turtles are in the immediate vicinity, where they might be startled.

Prior to any animal capture the tagging site will be evaluated for presence of environmental hazards that could present a risk of injury to the animal or the handlers. For example, seals will not be restrained or tagged if they are in proximity to rock ledges or hard substrate.

We will minimize stress from captures and restraint activities (for tagging, sampling, and instrumentation) by keeping the handling procedures as short as possible and cooling the animal with water. We believe that, for routine tagging which entails short restraint times, administering sedatives presents more of a risk to the seals than the stress, which the sedative would relieve, and the procedure would add significantly to the restraint time. Similarly, a local anesthetic such as lidocaine could be administered to relieve the transitory pain experienced by the seal when a tag is applied, but this would add significantly to the restraint time, presenting a higher risk.

Procedures requiring longer restraints such as biological sampling and instrumentation will involve the use of sedatives to calm the animal and reduce stress. Capture team always has a 5-10 minute briefing prior to the event to discuss roles of each team member, and contingencies/responsibilities in the event of unanticipated results or action by the animal. In the event of adverse reaction by the seal(s) to restraint, emergency procedures would be initiated under the advice of an on-site veterinarian. These procedures may vary depending upon the condition of the subject animal, but could proceed in the following order:

1) If respiratory arrest occurs, manual stimulation to restore breathing, including, as necessary, stimulation to face, chest compressions, intubation, and

administration of atropine and/or Dopram®. Dopram will be administered IV at dosage of 5 ml (pups/juveniles) and 10 ml (subadults/adults)

2) If cardio-vascular arrest occurs, administration of epinephrine by the most effective means (IV, IM, pericardial, and/or via airway) at a dosage of 1 ml/100-200kg. Dexathasone or solu-delta cortef may be administered after arrest to reduce shock.

3) If the emergency appears to result from diazepam overdose, Flumazenil may be administered to reverse the effects of diazepam. Flumazenil will be administered IV at a dosage of 2.5 ml (pups/juveniles) and 5.0 ml (subadults/adults), repeated if necessary. At the discretion of the veterinarian other medications may be administered, including sodium bicarbonate, physiological saline, aqueous dextrose solution, and lactated ringer's.

Restraint times for normal handling without sedation are very short (8-10 minutes). Because of this, if an animal is captured for instrumentation but is not sedated within 8-10 minutes, we will discontinue instrumentation and will release the animal immediately.

For translocations, whenever possible, seals will not be collected when other seals are in the immediate vicinity. In some instances, when an abandoned nursing pup is being sought, biologists will not take the pup if it is the vicinity of other seals, particularly mother/pup pairs. To introduce the pup to a parturient female, biologists will need to approach close to the female to place the pup in close proximity to the female, which will result in brief harassment to the adult. Generally a single person does the placement quickly.

For adult male removals, during all restraint procedures, seals will be sedated to reduce stress during handling. Seals that are transported will be kept wet throughout all daylight hours, but will be otherwise undisturbed. If seals are euthanized, sodium pentobarbital is a means of administering euthanasia generally accepted by veterinarians. The proposed action will result in the affected animals being permanently removed from the Northwestern Hawaiian Islands, and possibly will result in the deaths of the individuals. In all cases, the least intrusive alternative of our three options will be used. The activity will be submitted to the University of Hawaii IACUC for review and approval.

Biologists will attempt to remove entangling items without restraint (i.e., by using a long-handled cutting implement) whenever possible. If restraint is necessary, seals will be cooled with water if restraint is not near the waterline and is during midday times.

As described in the project description, behavioral modification techniques will be applied carefully and judiciously when needed for protection of individual seals and the public. Aversive stimuli that may be used would be tested carefully and while they may cause discomfort or annoyance, would not cause physical harm to seals.

Biologists will remain out of sight of seals and turtles to the extent possible while still accomplishing research and enhancement objectives as listed in this application. Nesting

seabirds on the beach will be avoided. Boats transiting lagoons will not divert from straight line paths necessary to transit between the islands, and boats will avoid landing on beach areas where seals or turtles are in the immediate vicinity. Campsites at islands where Laysan finches are present will be inspected regularly for presence of hazards to the birds.

Seals are observed for a minimum of five and up to 20 or more minutes after being marked, disentangled, or handled for any reason (tagging, sampling, instrumentation involving sedation) to ensure they resume normal behavior (either going into water or resuming normal respiration rates on land). Seals going into the water will be observed until they are out of sight.

Seals are observed for five minutes after being bleach marked to monitor their behavior and assess the likely efficacy of the mark—bleach is not likely to have a good effect if the animal goes into the water shortly after marking, or the animal may roll onto the mark before the bleach takes, rendering the mark illegible. Follow-up sighting records are maintained of marked seals throughout the field season.

Regular patrols and censuses of the area will be conducted to resight animals that have been moved. For attempted (re)introduction of nursing pups to parturient females, biologists will observe until it becomes clear whether the prospective foster mother accepted or rejected the pup.

At least a subset of translocated seals will be instrumented with tracking devices that will allow for a period of post-release assessment of location, behavior and survival.

Effects to Non-Target Species and Mitigation

Non-target animals may be incidentally disturbed during routine field camp activities, small boat operations, and research activities directed at Hawaiian monk seals. Effects to non-target species are presented in Appendix H.

Resources needed to accomplish objectives

Cooperators and contractors include the State of Hawaii Department of Land and Natural Resources, the Research Corporation of the University of Hawaii, Aquatic Farms Ltd., the U.S. Fish & Wildlife Service, the NOAA Office of Marine and Aviation Operations, and the NOAA Papahanaumokuakea Marine National Monument.

Agencies/cooperators that will be receiving specimens for analyses and/or curation include the following:

Colorado State University
Diagnostic Laboratory
College of Veterinary Medicine
300 West Drake
Fort Collins, CO 80523

Dalhousie University
Dept of Biology
Halifax, Nova Scotia
Canada B3H4J1

IDEXX Veterinary Services
2825 KOVR Drive
West Sacremento, CA 95619

NOAA, National Ocean Service
219 Fort Johnson Rd
Charleston, SC 29412

Oklahoma Animal Disease Diagnostic Lab
Farm Road and Ridge
Stillwater, OK 74078

USGS-NWHC-HFS
PO Box 50167
300 Ala Moana Blvd. Room 5-231
Honolulu, HI 96850

National Wildlife Health Center
6006 Schroeder Rd.
Madison, WI 53711

National Veterinary Services Laboratory
1800 Dayton Ave.
Ames, IA 50010

The Marine Mammal Center
Golden Gate National Recreation Area
Sausalito, CA 94965

CAHFS Thurman Lab West Health Sciences Dr.
University of California-Davis
Davis, CA 95616

Marine Ecosystem Health Diagnostic and Surveillance Laboratory
VM: Wildlife Health Center
TB 128 Old Davis Rd
University of California
Davis, CA 95616

Washington Animal Disease Diagnostic Lab
Washington State University
College of Veterinary Medicine
Bustad Hall, Room 155-N
Pullman, WA 99164

10300 Edmonston Road
Bldg. 1001, BARC-East
Animal Parasitic Diseases Lab
ANRI, BARC, ARS
U.S. Department of Agriculture
Beltsville, MD 20705-2350

Athens Diagnostic Laboratory
College of Veterinary Medicine
University of Georgia
Athens, GA 30602

Mystic Aquarium
Department of Research and Veterinary Services
55 Coolgan Blvd
Mystic, CT 06355

Molecular Parasitology Unit
Laboratory of Parasitic Diseases
National Institutes of Allergy and Infectious Diseases
4 Center Drive, Rm B1-06
National Institutes of Health
Bethesda, MD 20892-0425

Sackler Institute of Comparative Genomics
American Museum of Natural History
W. 79th St. @ Central Park West
New York, NY 10024

Cornell Stable Isotope Lab
E440 Corson Hall, Tower Road
Ithaca, NY 14853

School of Veterinary Medicine
University of Wisconsin
2015 Linden Drive
Madison, WI 53706

Diagnostic Virology Laboratory
National Wildlife Health Center
United States Geological Survey
Department of the Interior
6006 Schroeder Road
Madison, WI 53711

Mote Marine Laboratory
1600 Ken Thompson Parkway
Sarasota, FL 34236

Northwest Fisheries Science Center
Environmental Conservation Division
2725 Montlake Blvd. E
Seattle, WA 98112-2097

Dave S. Rotstein, DVM, MPVM, Dipl. ACVP
NOAA Center for Marine Animal Health
Assistant Professor, Department of Pathobiology
College of Veterinary Medicine
University of Tennessee
2407 River Drive, Room A201
Knoxville, TN 37996-4542

Bigelow Laboratory
180 McKown Point Road
West Boothbay Harbor, ME
04575

University of Florida
College of Veterinary Medicine
2015 South West 16th Avenue
Building 215
Room VC 65
Gainesville, FL 32608

Disposition of tissue samples
Tissue samples that are not consumed in the analysis process are retained by the HMSRP. The Hawaiian Monk Seal Health Program maintains these samples and a database that documents their provenance, analysis and current location. In some cases, samples may be archived with collaborators (e.g., USGS, NIST).

Publication of results
Data summaries of all NWHI field monitoring are typically available within 3 months of completion of the field season, and MHI summaries are provided at annual Recovery Team meetings. Draft Stock Assessment Reports are updated annually, typically in November. Publication of research results in peer-reviewed journals (see references above) is an ongoing process.

Project Locations
The project would encompass the range where Hawaiian monk seals are found throughout the Hawaiian Archipelago and Johnston Atoll including the NWHI and MHI (Figure 1). More specifically, the project would include portions of the open ocean and nearshore environment where monk seals may be found as well as the shorezone of the islands, islets and atolls that make up the Hawaiian Archipelago and Johnston Atoll. For the purposes of this project, the shorezone includes terrestrial habitat 5 meters inland from the upper reaches of the wash of the waves, at high tide during the season in which the highest wash of the waves occurs, usually evidenced by the edge of vegetation growth or the upper limit of debris.

In the NWHI, monk seals have six main reproductive sites including Kure Atoll, Midway Islands, Pearl and Hermes Reef, Lisianski Island, Laysan Island, and French Frigate Shoals. Necker and Nihoa Islands have smaller breeding sub-populations and monk seals have been observed at Gardner Pinnacles and Maro Reef. Monk seals are also found throughout the MHI.

Biological samples may be exported/re-imported for analysis, per list of cooperating agencies below. Currently the only cooperators are in Canada. We request export to any country (world-wide) in the event additional cooperators are identified to run analyses. All exports will be from Honolulu, Hawaii, a U.S. designated port. Samples of Mediterranean monk seals (*Monachus monachus*) may be imported/re-exported for analysis related to the conservation of monk seals. CITES permits will be obtained as necessary for all exports.

Protected areas—A large part of the proposed activities will occur within the Papahanaumokuakea Marine National Monument (PMNM), and will require appropriate permitting by co-trustees of PMNM. Permitting by the co-trustees provides appropriate authority to operate in the following special areas which are overlaid by the PMNM: Hawaiian Islands National Wildlife Refuge, the Midway Atoll National Wildlife Refuge, Battle of Midway National Memorial, Northwestern Hawaiian Islands State Marine Refuge, Kure Atoll Hawaii State Seabird Sanctuary, and the Northwestern Hawaiian Islands Coral Reef Ecosystem Reserve.

Activities in the MHI will occur in the following special areas under jurisdiction of U.S. Dept. of Interior, National Park Service (NPS): Kalaupapa National Historical Park (Molokai), Volcanoes National Park (Hawaii), and Haleakala National Park (Maui). Permits from the NPS will be obtained as necessary.

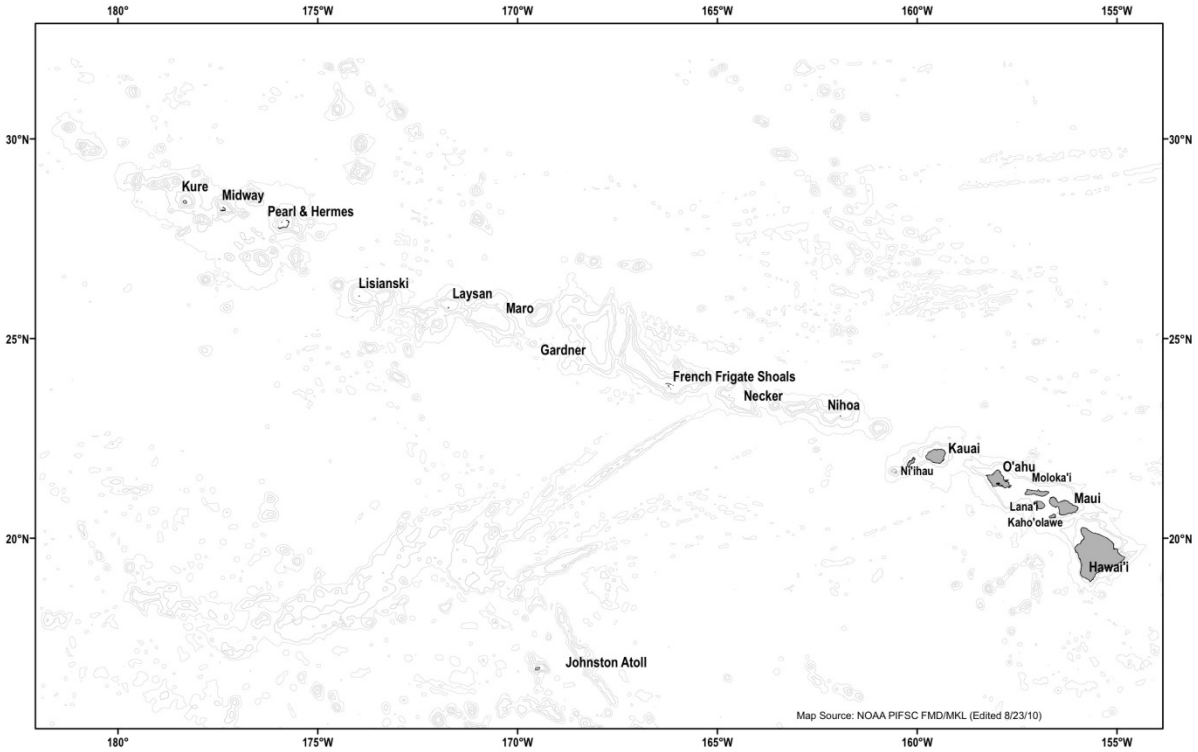
Activities in the MHI will occur in the following special areas under jurisdiction of the State of Hawaii, Department of Land & Natural Resources (DLNR): Manana (Rabbit) Island, Mokolua Islands, Kaena Point Natural Area Reserve. Permits or permission will be obtained from Hawaii DLNR as necessary.

Activities in the MHI will occur in the following military areas: Kaneohe Marine Corps Base Hawaii; Pacific Missile Range Facility, Kauai (including Kaula Rock). Permission will be obtained from appropriate military authority as necessary.

Johnston Atoll is a National Wildlife Refuge under U.S. Fish and Wildlife Service, and as such any observations there will require and be conducted under a Special Use Permit issued by USFWS.

Monitoring activities in all areas will occur on the beach or nearshore lava bench areas of all specified locations. Beaches in all NWHI (except Sand Island, Midway) are part of Critical Habitat for the Hawaiian monk seal.

Figure 1. Hawaiian Archipelago and Johnston Atoll



National Environmental Policy Act (NEPA) Considerations

NMFS has prepared a Draft PEIS on Hawaiian monk seal Recovery Actions to assess the environmental impacts associated with NOAA implementing the alternative approaches for undertaking and permitting the research and enhancement activities on Hawaiian monk seals. The Draft PEIS provides a more detailed analysis of the following and is available on NMFS's web site: http://www.nmfs.noaa.gov/pr/permits/eis/hawaiianmonksealeis.htm.

1. **New Techniques:** The techniques involved with the proposed activities are not new, but some may be newly implemented for monk seals, such as vaccinations and 2-stage translocations.

2. **Pathogens**: Chemical fixatives and preservatives are taken to the field sites for specimen collection. Transport and storage of all such materials are conducted in accordance with OSHA regulations, and Material Safety Data Sheets are present at all field camps.

3. **Unique geographic areas—altering substrate:** Anchorages of small boats incidental to conducting the activity are selected with consultation with PMNM co-trustees, and will not adversely affect substrate. Field camps established to conduct monitoring activity are transient and will not alter the substrate. All trash or other disposable material is removed from the island upon completion of the field season. Latrines are dug to a depth of at least 10 feet for human waste, and are filled in at the end of each season.

4. **Historical or cultural resources—**The Draft PEIS addresses historical and cultural resources. NMFS is preparing additional analyses on impacts to historical and cultural resources, which will be available in the Final PEIS.

5. **Transport of animals, ballast water discharge, etc.:** All small boat hulls are inspected for presence of invasive species prior to deployment into the field. Moreover, strict quarantine procedures are followed to prevent introduction of invasive species to non-native areas. These procedures include packing of all materials into plastic buckets when possible, freezing for 48 hours any soft materials which cannot be placed in buckets, use of clothing which is either new off the shelf or which has only been worn at the site to which the particular biologist is stationed, and fumigation of sensitive electronic equipment which cannot be frozen or packed into buckets. Regarding movement of Hawaiian monk seals between locations, approximately 10% of a subpopulation may move between islands annually, so we will not be moving any animals with a risk of introduction of novel pathogens. Permits issued by the Monument to access the NWHI require such stringent controls to prevent introduction of invasive species.

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