

File No. 16632 Appendix A: Two-Stage Translocation Plan

Two-Stage Translocation: A Proposal for Enhancement of the Endangered Hawaiian Monk Seal¹

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¹ An earlier version of this document was prepared for a Society for Conservation Biology (SCB) blue ribbon panel review of the science supporting two-stage translocation. Some of the comments and suggestions arising from the SCB review (completed 7 February 2011) have been incorporated into the current version of this document. Other suggestions, such as providing a wider range of metrics for evaluating two-stage translocation benefits, were incorporated directly into Chapter 4 of the PEIS.

Context and Scope

The National Marine Fisheries Service (NMFS) is proposing a novel strategy for boosting juvenile Hawaiian monk seal survival. The proposal involves temporarily translocating weaned female pups from subpopulations with relatively low juvenile survival to alternate sites where juvenile survival is much higher, then returning them several years later. The objective is to reduce early mortality of these individuals, which is exceptionally high in the first two years of life and is thought to be the primary factor limiting population recovery. The proposed translocations would ideally preserve sufficient reproductive potential within monk seal subpopulations maintaining the capability for more rapid growth should conditions currently constraining survival eventually relax. Given recent trends for this species (4% annual decline in abundance), this logic is admittedly optimistic, but some improvement in natural survival will surely be required if the species is to avoid extinction.

Current survival rates suggest the most favorable option (purely in terms of demography) would involve temporarily moving seals from the remote Northwestern Hawaiian Islands (NWHI) to the main Hawaiian Islands (MHI), an initiative that would undoubtedly involve some controversy related to socio-economic issues. A draft Programmatic Environmental Impact Statement (PEIS) to support this proposal as well as other recovery actions was made available to the public in September 2011.

As described below, the proposed translocation program is but one of several actions, currently underway or proposed, to conserve the Hawaiian monk seal. All of these actions have been, or will soon be, subject to scrutiny for NEPA clearance, MMPA/ESA permitting, IACUC approval, and Recovery Team and Marine Mammal Commission review. Most of these activities have a long history of positive application to monk seals or demonstrated precedent in other wildlife management or conservation programs.

In contrast, the proposed translocation program is novel in many respects and deserves special consideration. Social and economic concerns associated with translocations will be thoroughly analyzed and addressed during the PEIS and permitting processes. However, the PIFSC has further commissioned this special Society for Conservation Biology (SCB) review of the science of its proposed translocation strategy. The PIFSC recognizes that the proposed two-stage translocation program has unique features in terms of its design, execution and underlying scientific principles when compared to 'traditional' translocation or reintroduction programs. As such, the SCB review is intended to evaluate the scientific support for the proposed strategy. While recognizing that the translocation program would occur as one element of a more comprehensive research and enhancement program, the scope of this review is relatively narrowly focused on translocation science.

Background

Distribution and Population Status

The Hawaiian monk seal ranges throughout the entire Hawaiian Archipelago with rare occurrences recorded at Johnston Atoll, approximately 800 km south of Hawaii (Figure 1).

The species is structured in a metapopulation consisting of eight NWHI subpopulations, which together comprise roughly 85% of total abundance; the remainder is distributed amongst the MHI. The monk seal subpopulations display varying degrees of demographic independence but are linked through regional environmental correlation as well as migration (Baker *et al.* 2007, Baker and Thompson 2007, Schultz *et al.*,2011). A proxy for movement rates among subpopulations (the proportion of tagged seals seen at other than their natal site during their lifetime) ranges from 4% to 18% depending upon the site (Schultz *et al.*,2011). Effective migration has apparently been sufficient to preclude any discernable genetic population structure, such that the species is comprised of a single panmictic population (Schultz *et al.* 2009, Schultz *et al.*,2011).

Total Hawaiian monk seal abundance is approximately 1,100 individuals with subpopulations ranging from roughly 50 to 200 seals each. The overall population abundance is falling by an estimated 4% per year. The six most-studied subpopulations in the NWHI (French Frigate Shoals, Laysan Island, Lisianski Island, Pearl and Hermes Reef, Midway Atoll and Kure Atoll) are currently declining with estimated intrinsic rates of increase (λ) ranging from 0.89 to 0.96 (Baker *et al.* 2011a). Necker and Nihoa Islands appear to be stable or increasing, however the demographics at these two sites are relatively poorly characterized due to their difficult access and historically relatively small contribution to total abundance. In contrast, the MHI population is increasing with an estimated λ of 1.07.

Poor post-weaning juvenile survival is the primary driver of the population decline in the NWHI and, conversely, favorable survival in the MHI contributes to that region's robust growth. Recent survival to age curves (l_x) demonstrate the divergent survival regimes operating between the NWHI and MHI (Figure 2). Chronic poor juvenile survival for time periods ranging from 10-20 years in the NWHI have resulted in degraded age structures exhibiting an over-representation of newborns and older seals, with few juveniles and young adults.

Age-specific fecundity (m_x) has been rather well characterized for three NWHI subpopulations (Harting *et al.* 2007, Figure 3). The curves vary among these sites and tend to be somewhat lower than for other pinnipeds. There is some evidence that MHI seals enjoy earlier maturation and higher reproductive rates, at least among the younger adults (Baker *et al.*2011a). Nevertheless, survival rates are the primary factor determining population status and trends at present.

Causes of population decline

The 2007 Recovery Plan for the Hawaiian Monk Seal (NMFS 2007) identified three "crucial" threats to the species:

- **Food limitation**, the primary cause of low juvenile survival.
- **Entanglement** in marine debris, which affects all ages and sexes, but disproportionately involves juvenile seals.

- **Shark predation**, particularly Galapagos shark predation on pups at French Frigate Shoals.

Another set of second tier “serious” threats include infectious disease, terrestrial habitat loss in the NWHI (especially due to sea level rise), intra-specific male aggression, and human interactions especially in the MHI (disturbance, fishery interactions, etc.).

While certain of these threats can have important sporadic or localized impacts (*e.g.*, male aggression) or have *potential* for widespread, devastating impacts (epidemic disease), it is generally agreed that the primary cause of the current decline is food limitation leading to unsustainably high levels of juvenile mortality (Antonelis *et al.* 2006, Baker 2008). Insufficient availability of prey for young seals may be mediated through poor or variable overall system productivity, competition with other top predators (Baker *et al.* 2007, Polovina 2008, Baker and Johanos 2004, Parrish *et al.* 2008), or both. In any case, because the diagnosis indicates a deficiency in the ecosystem that is leading to the demise of young monk seals, there are no simple or certain remedies. Thus, a set of novel tools, including a new translocation approach, is being proposed. Below we describe past, ongoing and future planned interventions to provide some context for the translocation proposal that is the focus of this review.

Past and current enhancement activities

Due to steep declines in abundance following surveys in the late 1950s, the Hawaiian monk seal was listed as endangered under the United States Endangered Species Act (ESA) in 1976. Efforts to monitor the species and foster its recovery began in the early 1980s, led by the NMFS as prescribed by the ESA. Monk seal population assessment has focused on determining abundance, age and sex structures, survival rates, reproductive rates, and causes of injury and mortality. The Hawaiian monk seal thus has the distinction of being the subject of a long-term and thorough demographic study on a par with that undertaken for any large, free-ranging mammal in the world. Relying on the rich data set accumulated from over two decades of research, a suite of demographic parameter estimates has been updated annually for six NWHI subpopulations, with less data available from Necker and Nihoa Islands, and more recently, data from the MHI. Summarized demographic data are typically available for review within a few months after annual field seasons have ended. Further, robust investigations of foraging behavior and monk seal health and disease are ongoing. This rich, two-decade plus research data set is essential for evaluating past recovery efforts and designing future measures. A primary focus of the research program has naturally been to discover and, when possible, mitigate natural and anthropogenic threats to the species.

Future proposed interventions

Despite the many past efforts and those ongoing, the monk seal’s status continues to erode. The efforts outlined above have no doubt slowed the species’ decline, but it is broadly agreed that more must be done to save the species from further deterioration and ultimately, extinction. Because the primary driver of decline is low juvenile survival,

successful interventions must be directed toward the early life stages: pups and juveniles. However, due to the condition of age structures and vital rates in the NWHI as described above, the number of pups available for intervention is projected to rapidly decline (Figure 4). This realization heightens the sense of urgency to begin interventions before the opportunity to effect meaningful improvement expires.

Many past and current efforts will be continued into the foreseeable future as these measures have clear and direct benefits. These include, but are not limited to, disentangling seals caught in marine debris, removing fishing hooks from seals, large-scale removal of potentially entangling marine debris from beaches and reefs, and mitigating Galapagos shark predation and intra-specific male aggression when needed. Some translocations, already authorized, will continue. For example, within-atoll translocation of weaned pups from high shark predation islets to historically safer islets at French Frigate Shoals is a successful tool for mitigating post-weaning Galapagos shark predation. In the MHI, pups that wean in high human-use areas isolated from other seals may also be translocated to more favorable sites when deemed beneficial. Finally, translocation of adult males is one option authorized for mitigating male seal aggression.

The robust Hawaiian monk seal research effort will continue and expand in the future. This program is focused on four broad areas: population monitoring, foraging ecology, health studies and survival enhancement research. The full details of the research program are beyond the scope of this document, but it is important to recognize that each element of research inquiry is integrated into the goal of species' conservation. Investigations serve to identify threats, characterize underlying factors that influence survival and reproduction, design interventions, and evaluate the success of conservation measures.

Coupled with the research program is an expanding management effort, primarily focused on the MHI. The management program, led by the NMFS Pacific Islands Regional Office entails stranding response, public outreach and education, and legal/regulatory issues.

Another anticipated expansion is in the area of captive care of monk seals. In collaboration with the Marine Mammal Center in Sausalito, NMFS is pursuing expanded capacity for captive care facilities. Care would be provided to seals brought into temporary captivity under the authority of the NMFS Marine Mammal Health and Stranding Response Program. Captive care efforts would be limited to animals deemed in need of medical intervention.

In addition to the foregoing measures, a set of new research and enhancement tools is under consideration to promote recovery of the Hawaiian monk seal. These include:

- Two-stage translocation
- De-worming
- Vaccination research
- Behavioral modification

The proposed two-stage translocation program is the subject of this paper and SCB review, however the other three initiatives will be described briefly.

De-worming is currently being investigated as a means for improving free-ranging juvenile seal survival by temporarily reducing gastrointestinal parasite burden. If this approach is determined to be feasible and effective, it may be used as an enhancement tool.

Vaccination research is meant to address potential disease (*e.g.*, morbilliviruses and West Nile Virus) outbreaks that could devastate Hawaiian monk seals. If the safety and efficacy of specific vaccines are established, then these could be used either prophylactically or as a response tool to contain an outbreak.

Behavioral modification research addresses a range of measures primarily intended to prevent or mitigate human-seal interactions. Occasionally seals become socialized to humans in the MHI and because of the dangerous nature of their interactions with people, these seals have typically been translocated from the MHI or brought into permanent captivity. Seals also interact with fishers, sometimes to the detriment of the former (hooking, entanglement, shooting) and the latter (loss of catch, damaged gear). Tools to prevent or alter such behavior will be in greater demand as the MHI monk seal population continues to grow. As the tools and protocols for effective behavior modification are refined, they will become an integral component of monk seal management in the MHI.

Two-stage Translocation

Basic concepts

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.* 2011b). The NMFS is proposing a novel approach to further apply translocation to enhance the Hawaiian monk seal population. Translocating individuals would have one or more of the following objectives:

- 1) Increase individual fitness (especially survival).
- 2) Improve the species status (*e.g.*, abundance, population reproductive value).
- 3) Maintain meta-population structure for long-term resiliency.

The fundamental concept underlying application of translocation is to address mismatches between local environmental conditions and distribution of seals among subpopulations. For example, some pups wean at subpopulations where they experience high mortality, apparently largely due to insufficient prey resources. Thus, many of these neonates perish, whereas, because of spatial variability among sites, they might have survived elsewhere. This would be tolerable under different conditions. That is, if the monk seal population were large and if mean environmental conditions were more favorable (although still punctuated with periods of unfavorable conditions), the meta-population might achieve a sort of dynamic stability across the entire range. The current situation, however, is not sustainable because the number of monk seals is perilously low and steadily declining.

Further, adverse conditions have largely prevailed for a decade or more, and natural dispersal occurs at far too slow a rate to effect a more optimal distribution.

Translocation, then, is a tool that could mitigate population decline by accelerating dispersal of young animals from areas of low survival (referred to as “donor” or “natal” sites) to areas of higher survival (referred to as “recipient” or “nursery” sites). This approach could achieve objectives 1 and 2 above. Nonetheless, if translocations are conducted at an appropriate scale for a sufficient number of years, some potentially negative consequences must be addressed. For example, donor populations may become unacceptably depleted or exhibit skewed sex ratios (as only females will be selected for translocation). Moreover, moving too many seals to recipient sites might result in overcrowding and adversely impact vital rates. For these reasons, some translocation measures will also be taken to achieve objective 3 above.

The proposed two-stage translocation approach is illustrated by the following. The NMFS Pacific Islands Fisheries Science Center (PIFSC) currently holds a permit to translocate weaned pups among NWHI subpopulations to improve their probability of survival. Unfortunately, all the primary NWHI subpopulations are experiencing relatively low juvenile survival (Figure 2) such that the potential efficacy of translocation amongst those subpopulations is uncertain. However, present conditions are favorable in the MHI, suggesting that the greatest positive effects of translocation could be achieved by moving weaned pups from the NWHI to the MHI. While juvenile survival in the NWHI is low, those seals that reach adulthood enjoy survival rates comparable to those in the MHI (Baker and Thompson 2007; Baker *et al.* 2011a). Thus, at present, the most effective scenario would likely involve moving weaned female pups from NWHI subpopulations to the MHI in order to increase the proportion surviving (first stage of translocation). Subsequently, animals that have achieved adult survival rate levels (*i.e.*, age 2 or 3 yr and older, following Baker and Thompson 2007 and Baker *et al.* 2011a) would be returned from the MHI to their natal NWHI subpopulations (second stage translocations). The latter action will serve to rebalance population distribution to avoid excessive depletion of donor subpopulations, ensure the MHI does not become over-populated, and prevent problems associated with male-biased sex ratios at donor sites. Further, should environmental conditions become more favorable in the future, this return translocation would serve to fortify subpopulation age structures, positioning them to exploit improved conditions and achieve positive growth. Without the second stage of the translocation process, donor subpopulations would likely become sufficiently depleted from prolonged low recruitment that population growth would be very slow, even in newly favorable environmental conditions.

It must be emphasized that while the preceding translocation scenario (*i.e.*, NWHI to MHI and return) is suggested by current conditions, future conditions may well dictate other approaches. For example, when juvenile survival is sufficiently high at any NWHI subpopulation, these NWHI subpopulations might be considered for receipt of translocated weaned pups. Likewise, if MHI conditions deteriorate significantly in the future, moving weaned pups from the MHI to the NWHI might be beneficial. Thus, it is critical to underscore that while the underlying translocation strategy is consistent, the particulars will necessarily be adaptive in accordance with prevailing monk seal demographics and

environmental conditions. Furthermore, the realized success of translocations is uncertain. Because of the dynamic state of the system and the uncertainty of outcomes, the translocation program would be guided by a complex and adaptive decision framework.

Genetic considerations

Strong genetic population structure can imply local adaptation across a species' range. When planning translocations in such a context, the risk of diluting local adaptation is of critical importance. In contrast, the Hawaiian monk seal's lack of population structure coupled with observed levels of natural movement amongst subpopulations indicate that translocations may be conducted without fear of genetic consequences (Schultz *et al.* in press).

Decision framework

A host of complex and interacting issues arise from three fundamental features of the proposed translocation program:

- 1) The program will, by design, occur over a span of several years.
- 2) Environmental and, perhaps in smaller subpopulations, demographic stochasticity lead to variable and unpredictable monk seal survival rates over time and space.
- 3) This is a novel recovery strategy the outcomes of which are uncertain, and there is potential for unintended (including undesirable) outcomes.

The remainder of this document focuses on the design, execution, and evaluation of two-stage translocation supported by a decision framework and simulation modeling. The decision framework and modeling reflect an attempt to consider all relevant inputs to inform actions and foresee and minimize the risks of undesirable translocation outcomes.

The critical importance of the accumulated monk seal demographic database and the continued stream of annual monitoring data cannot be over-emphasized. Existing survival and age/sex structure information will be the primary basis for determining when to conduct translocations and between which subpopulations. Continued monitoring of both translocated and non-translocated individuals will provide the basis for project evaluation, informing the subsequent steps and reducing uncertainties of simulations.

The skeleton of the decision framework is depicted in two flow charts, one for each stage of translocation (Figure 5). A narrative follows, which travels through each step in the flow charts. Next, explicit risks of undesirable outcomes are described and components of the decision framework that mitigate those risks are presented.

Translocation of weaned female pups (Figure 5a)

The flow charts in Figure 5 are color-coded to help illustrate the decision-making process. Green boxes represent decision points or actions that progress toward translocation, whereas orange boxes indicate circumstances where translocations are suspended. Yellow

boxes represent information inputs that influence decisions. Lastly, red numbers serve as references for orienting the following narrative with the chart.

Step 1 (in Figure 5a) is to evaluate whether there is a “substantial and consistent” difference in juvenile survival between at least two subpopulations. This indeed is the primary motivator for the entire translocation scheme. The two elements of this evaluation, “substantial” and “consistent” require further explication.

The magnitude of the difference in survival suggests a maximum expected benefit that could be conferred by translocation. For example, if survival for a given age class at two hypothetical subpopulations were 0.30 at site *a* and 0.70 at site *b*, then at best we could anticipate a 0.40 (0.70-0.30) improvement in the survival of seals moved from site *a* to *b*. The greater the survival differential, the more compelling the case is for translocation. However, establishing a concrete threshold for when translocation is worth doing is problematic, because we have insufficient experience with this intervention approach to reliably anticipate outcomes. Nevertheless, we require some guidelines to begin with, which will be refined as experience accumulates. The earliest age when translocations might occur is at weaning, and monk seals tend to achieve adult survival rates at approximately age 3 yr. Thus, an appropriate period for comparing survival amongst subpopulations is from weaning to age 3 yr. Initially, we will examine survival for this period among subpopulations but not hold to thresholds, which would be arbitrary if established *a priori*. While it could be argued that any improvement in survival is valuable, no matter how small, potential decrements to survival associated with translocation (see simulation modeling section) might subtract from the expected benefits of being placed in a more favorable environment. For initial trials the survival differential will be sufficiently large to allow the potential for considerable survival decrements to translocated seals without the action causing harm (*i.e.*, improvements should exceed decrements).

The concept that differential survival should be consistent before translocation is warranted arises from the observation that juvenile monk seal survival rates are notoriously variable among sites and from year to year. Previous analysis has shown that there is only weak autocorrelation in first year survival between years, such that poor survival in one year does not provide much predictive power about the next cohort’s survival prospects (Baker and Littnan 2008). Not only do survival rates fluctuate, but estimates have associated error, in part because the cohort size at individual sites can be very low. In order to avoid having our translocation decisions constantly chasing last year’s rates, we propose evaluating survival differential using the most recent available three years at each site. As with the magnitude threshold, this approach will be refined as information on outcomes is collected.

Thus, in Step 1, using the stochastic simulation model described in subsequent sections, we evaluate whether there is a sufficient differential in survival from weaning to age 3 yr measured over the past three years among subpopulations. If not, then continued monitoring of vital rates (**Step 2**) is prescribed. If yes, then we proceed to **Step 3**.

At **Step 3**, we ask whether the project has been ongoing for at least 2 years. If not, there are not yet any candidates for the return translocations, so we proceed directly to **Step 6**. However, if the project has been conducted for at least 2 years, we evaluate **Step 4**, whether return translocations of 2+ yr-old seals previously moved as weanlings are occurring as planned. Examples of conditions which might result in failure to return seals as planned would be an emerging concern about a pathogen affecting either subpopulation, unanticipated logistical problems or other factors as described below. If seals are not being returned as planned, then weaned pup translocations are suspended (**Step 5**) until whatever is impeding return translocations is resolved. This decision is intended to both avoid overloading a recipient site with immigrants and preventing over-depletion and sex ratio imbalance at donor sites that are not being replenished.

At **Step 6**, the donor and recipient subpopulations are determined. This will typically be a simple matter of selecting the two sites with the lowest and highest survival, respectively. However, there may be cases where more than one site has similarly low or high survival, such that weaned pups could be drawn from or delivered to more than one site. As in Step 1, simulation modeling will be conducted to evaluate expected benefits associated with selecting various combinations of donor and recipient sites. If weaned pups have been translocated to the proposed recipient site in recent years, the survival performance of the former translocatees will inform this decision.

Step 7 is a critical juncture where the number of seals to be translocated is determined. This decision is influenced by numerous factors indicated by the yellow boxes. The *smallest* number indicated by any of these factors should be the *maximum* number considered for translocation. For example, the “number of weaned female pups in healthy condition” at the prospective donor site sets a clear upper bound on the potential number available for translocation. Likewise, logistical constraints (ship deck space, ship availability, funding, etc.) might also limit the number that can be translocated. Further, the number deemed prudent to translocate in any one year may be influenced by societal factors (especially in the MHI). Regardless, when the program is new, it will be prudent to start small with approximately 5 weaned pups, gradually increasing to at most 10 per year in the first several years. Finally, the capacity for the prospective recipient sites(s) to absorb a cadre of additional weaned pups must be considered. This will largely be assessed by evaluating trends in juvenile survival. For example, first year survival post-weaning appears to be sensitive to worsening conditions. Thus, if a trend towards deteriorating survival is observed, this would suggest translocating fewer numbers of new pups. Lastly, social factors (public attitudes) may indicate that receiving sites within the MHI can absorb fewer additional seals than might be concluded on biological grounds alone.

Once the target number is determined, seals will be captured at their natal sites (**Step 8**) and screened for a variety of health parameters including indications of infectious disease (**Step 9**). Health screening protocols evolve with techniques and perceived potential for specific diseases. However, PIFSC has established protocols for health screening translocated weaned pups, which are periodically reviewed and which have been applied as recently as 2009. Seals which do not pass the health screen will either remain at liberty at the natal site or will be brought into captive care if deemed in need of medical attention

(Step 10). Those that pass the health screen will be transported to their destination, released, and closely monitored (initially with telemetry) **(Step 11)**. Past experience has shown that direct release of weaned pups in appropriate habitat (*i.e.*, at sites where other pups have previously been weaned and survived) is a successful strategy (Baker *et al.* 2011b).

Translocation of seals age 2 yr and older (Figure 5b)

The second stage of the proposed translocation involves repatriation of seals, previously translocated as weaned pups, which have achieved adult survival rates (2 or 3+ yr-olds). The precise age when young seals achieve adult survival rates is not fixed and may depend on factors such as their body condition at weaning and environmental conditions where they spend their first few years of life. The optimal age for returning seals is therefore not known, but will be informed by experience as the translocation program is conducted. Thus, some previously translocated seals may be returned at age 2 yr, but all would be slated for return by the time they reach age 3 yr. Figure 5b depicts the flow chart for the return translocation, with color-coding and notation conforming to that in Figure 5a.

Step 1 is reached when translocations have occurred two years or more previously, so that there are potential translocatees available for repatriation. At **Step 2**, we assess whether the survival prospects for 2-yr-olds, 3-yr-olds and adults in the seals' natal region are roughly as high or higher than in the current location. The reasoning here is that while juvenile survival varies greatly among subpopulations, adult rates tend to be more similar and less variable. For example, although juvenile survival is currently much lower in the NWHI than in the MHI (Figure 2), adult survival in the NWHI is comparable or just slightly lower than that in the MHI (Baker *et al.* 2011a). Thus, the two-stage translocation effectively protects subjects from the high mortality they would have otherwise experienced as juveniles in their natal regions, and returns them at an age when they will likely experience relatively high survival. The two translocations, then, confer a net benefit on translocatees even if they experience slightly lower survival as adults when repatriated in their natal regions. The expected magnitude of this net benefit will be assessed using simulation modeling as described in subsequent sections.

Alternatively, if adult survival at the natal region is considerably lower, then return translocations would be suspended (**Step 3**) and additional weaned pup translocations from the donor population in question would also cease (see Figure 5a, **Step 5**). It is conceivable that in rare cases other factors might provide a compelling incentive for translocating 2+ yr old seals even if adult survival at the natal site is sub-optimal. For example, addressing an imbalanced sex ratio or some other deficit might influence the disposition of these young female seals. If adult survival at the natal region remains comparable to, or higher than, the current location, we proceed down the path to return previous translocatees to their natal region (**Step 4**). The number of age 2+ yr-olds to potentially return is simply determined as the number of surviving previously translocated weaned pups (**Step 5**). Based upon the body condition of individual seals and taking into account survival of any seals previously translocated at age 2 yr and prevailing survival

rates at the natal area, some 2-yr-olds may be returned. Again, all seals age 3 yr and older would be slated for return.

The next important decision is to confirm that returning seals to the site of origin is indeed appropriate and prudent at the present time (**Step 6**). This deliberation is influenced by multiple factors (yellow boxes). For example, if seals have been returned in previous years, the survival performance of those earlier returnees will be considered before additional seals are repatriated. More broadly, the capacity of the natal region to absorb returnees will be assessed as indicated by survival rates of all ages at the site, as well as current abundance relative to historical levels. Disease risk is another consideration. If a known disease is present at the natal subpopulation, but is absent from the seals' current location, then it would not be appropriate to expose returnees and thus risk their survival. If it is deemed inadvisable to return seals to the preferred (natal) location, then an alternate nearby location may be chosen, so long as that location is deemed prudent according to the above criteria. Finally, male-biased sex ratios have led to male aggression-related mortality in the past, and interventions to adjust sex ratio have successfully lowered this threat (Johanos *et al.* 2010). Thus, there may be cases where returning seals to a site, not necessarily their birth location, could be used to ameliorate male-biased sex ratios. If no appropriate release location is identified, then return translocations of 2+ yr-olds will be suspended (**Step 3**).

Once the release location(s) have been confirmed, the subject seals will be brought into captivity (**Step 7**, *in situ* pens/cages in the NWHI; permanent captive facilities in the MHI). At this point, the seals will be health screened as described above and also held in quarantine for a prescribed period; likely approximately two weeks, depending upon veterinary protocols to be developed (**Step 8**). The primary purpose of quarantine is to confirm absence of active disease and minimize the chance of transmitting a disease into a return site where that disease may be absent. The quarantine period may be shortened when moving animals between subpopulations where disease surveillance indicates that the prevalence of exposure to a suite of pathogens is equivalent. Quarantine is expected to be most important when moving seals from the MHI to the NWHI, as some diseases may occur in the former region but not the latter because of the presence of feral and domesticated animals in the MHI.

Seals which fail to pass the health screen or quarantine will be released at the capture site or brought into captive care if appropriate (**Step 9**). Otherwise, they will be transported, released and closely monitored (initially with telemetry)(**Step 10**).

Minimizing risk of undesirable outcomes

A variety of risks are inherent in any intervention in wild populations, including the proposed two-stage translocation. Risk minimization will be achieved through program design, intensive monitoring and evaluation, and the adaptive decision framework described above. Below, we address how the risk of an extensive list of conceivable potential ill effects will be minimized.

Table E-1. Risks and concerns that may affect the outcome and evaluation of two-stage translocations in Hawaiian monk seals.

Issue	Risk or Concern	Mitigating Factors
Condition of weaned pups (<i>e.g.</i> , axillary girth), is positively related to survival prospects.	Selection of weaned pups for translocation may not be representative (i.e only viable, healthy pups will be selected), so that project evaluation may be difficult.	Small, but otherwise healthy pups will not be excluded from translocation. Only non-viable, emaciated or wounded animals will be avoided. Post-hoc analysis will control for condition of both translocated and non-translocated pups.
Depletion of donor subpopulations.	If weaned pups are continuously taken from a site, abundance may fall to an unacceptably low level, with the potential that: i) Seals no longer play a “functional” role in the system. ii) Competitors may occupy the monk seal niche and inhibit population re-establishment. iii) “Empty” environment could be a wasted opportunity for growth if intra-specific competition is low.	Depletion should only be short-term and moderate because 2+ yr-olds will be returned to the donor population. This, in fact, should increase rather than deplete the donor population after return translocations commence. Moreover, should intra-specific competition lessen at the donor site, juvenile survival should consequently increase. This will reduce the survival differential between sites and automatically regulate further weaned pup translocations.
Development of male-biased sex ratios	Removal of female pups will eventually manifest in male-biased sex ratios, leading to increased male aggression toward adult females and juveniles.	Weaned female pups will be returned to natal sites prior to sexual maturity. Presumably they will have enjoyed higher survival than (non-translocated) males. Ultimately, the two-stage translocation should result in some female bias for effected cohorts. If in fact the translocated females fare poorer than their male counterparts or cannot be repatriated for any reason, weaned pup translocations would be suspended as described in the decision framework. This could result in male bias for a few affected cohorts, but this would be a small portion of the total population.
Capacity of recipient site to absorb immigrants.	Overshooting carrying capacity could lead to a crash of the recipient population.	Recipient site demographics will be closely monitored, especially for declining juvenile survival. If this is observed, the differential survival between donor and recipient sites decreases, so that translocations slow or cease, thus correcting the problem.
Translocated seal survival	Weaned pups taken from their natal sites may not fare as well as natives at their host site. Returned 2+ yr-old returnees may not survive as well as those who have survived from birth at their natal site.	Past experience (Baker <i>et al.</i> 2011b) has shown that recently weaned pups are amenable to translocation and have survival rates indistinguishable from pups born at release sites. Sites where pups have been weaned and survived will be selected as release locations for weaned translocation pups. Experience translocating juvenile seals is limited. Repatriates to their natal regions

		<p>may have both disadvantages and advantages relative those that have grown up there. Three-year-old seals may experience greater effect of capture stress than has been the case with weaned pups. Returnees may be disadvantaged by having to learn to forage in a new area, which may have less prey availability than where they grew up. However, because returnees spent their first 2 or 3 years in more favorable habitat, their body condition should be better than non-translocated seals in their natal region, thus providing a survival advantage.</p> <p>In both cases (weaned pups and returnees), survival will be monitored and translocation plans appropriately adapted as described in the decision framework.</p>
Infectious disease	Translocating seals may result in spreading disease faster than would occur naturally.	Health screening of all translocated seals, coupled with appropriate quarantine of returnees will minimize risk of transporting infectious agents. Moreover, disease surveillance will be ongoing throughout the species range to detect emerging disease outbreaks. At present, there does not appear to be strong differences in exposure throughout the range, perhaps with the exception of some diseases (leptospirosis, toxoplasmosis) more prevalent in the MHI than the NWHI.

Simulations to evaluate benefits from two-stage translocations

Model Design

The monk seal stochastic simulation model was used to compare and evaluate the expected outcomes from a representative set of translocation scenarios. Details of the model structure and mechanics are provided in Harting (2002) and only the fundamental features are described here. At its core, the model is a mechanistic, stochastic, metapopulation model with provisions for handling uncertainties in input parameters and modeled processes. The model is heavily data driven, capitalizing on the demographic and life history data collected over more than two decades in the NWHI and, more recently, the incipient demographic data set for the MHI. Necker and Nihoa Islands (NWHI) are relatively data poor and have historically comprised a small portion of total abundance, and are therefore not included in simulations. The model provides multiple options for simulating natural perturbations (survival catastrophes, birth catastrophes, shark predation, and aggressive male interactions) and management interventions (captive rearing/release, translocations, shark removals, and other). It produces a diverse array of outputs suitable for evaluating simulation outcomes including abundance, realized growth

rate, multiple demographic descriptors, and assorted metrics specific to whatever intervention scenario was executed. The primary output is site-specific, with summary diagnostics for the entire system and the two main regions (NWHI and MHI).

For the purposes of this analysis, certain model components were disabled, including the option for density dependent adjustment of demographic rates. While that feature of the model is certainly important when performing long-term projections, the precise manner in which density dependence operates on the monk seal population is unknown and its influence can overwhelm and obscure the effects of all other factors included in the simulation scenario.

For the NWHI, age-specific survival rates used for model input were derived from fitting the Siler survivorship curve to observed rates from the most recent three data years. Separate curves were fit for each of the 6 sites. For the simulations, parameter uncertainty was handled by random sampling Siler parameters from the variance/covariance matrix from the parameter fitting. Age-specific reproductive rates were estimated from pooling pupping data from 1990 to the present using methods described in Harting *et al.* (2007). As with survival rates, parameter uncertainty was handled by randomly sampling a unique set of correlated parameters from the fitted distributions. In the model, survival and reproduction are determined stochastically for each individual in the population by binomial sampling (testing a uniform random number in the range [0,1] against the age-specific survival rate). Migration is also determined stochastically for each individual according to the fitted movement rate for each age class. Each simulation was initialized with the most recent starting age/sex distribution for each NWHI site.

As compared to the NWHI, data from which to estimate vital rates and population composition are much more limited for the MHI. A detailed description of the methods used to fit both survival and reproductive rates for the MHI are provided in Baker *et al.* (2011a). Where data were lacking (*e.g.*, reproductive rates of older MHI females), some inference and extrapolation was necessary based on patterns observed in the NWHI. Uncertainty in parameter estimates was handled in the same manner as for the NWHI, with unique parameters drawn from their fitted distributions at the start of each simulation.

Translocation Scenarios

As described in the decision framework section of this document, the specific translocation scenario to be undertaken in a given year will be determined according to the most recent data available for each subpopulation. Results from preceding translocation efforts, logistics to accomplish the translocation and other considerations will also enter into the decision-making calculus. In a given year, the optimal translocation scenario might involve any combination of single or multiple donor and nursery sites. Further, the number of seals collected and translocated to each site will vary. It is not our intent to present and evaluate the full complement of translocation scenarios that might be undertaken, but rather to present a small set of representative scenarios that illustrate the salient aspects of this intervention strategy and highlight some of the variables and uncertainties that influence the expected outcome. In practice, prior to initiating an action, additional

simulations and ancillary analyses will be undertaken to inform NMFS about the relative benefits that might accrue from various translocation scenarios in a given year.

We present results from nine scenarios. These include one “baseline” scenario that involves no translocation and which serves as the basis of comparison for the other scenarios. This scenario is indicative of what would be expected if current vital rates remain applicable for the duration of the 10-year model projection, and no major perturbations or interventions alter the population trajectory.

The remaining simulations are divided into two sets of four simulations each: one set of cross-region translocations (from French Frigate Shoals (FFS) to MHI), and another set of within-NWHI translocations (FFS to Laysan Island (LAY)). These sites were selected primarily based on the current survival differential of the species’ main breeding sites as estimated from the most recent (2010) data. Considering only the NWHI, FFS has consistently had the poorest juvenile survival of any site ($l_3 = 0.137$), while LAY currently has had much better juvenile survival rates ($l_3 = 0.331$), although as with other NWHI sites, LAY has historically demonstrated considerable inter-annual variability (Figure 2). In contrast to all NWHI sites, the MHI has demonstrated the best juvenile survival of any breeding site ($l_3 = 0.641$).

For all scenarios, we simulated the collection of 10 female pups annually for 5 years at FFS and subsequent release at the nursery site (MHI or LAY). Although the model allows for mortality while in transport, for these simulations there was no deduction for captive mortality and the number of seals released was the same as the number collected. This is consistent with the very low levels of translocation mortality reported by Baker et al. (2011b). In actual translocations to the MHI, the specific island and release site will be chosen on the basis of past suitability for native pup survival as well as other (social) considerations. However, for purposes of estimating demographic rates, there is no distinction among sites in the MHI and hence the MHI release site was treated generically for the translocation simulations.

Once released, the translocated pups are presumed to merge with the native-born seals, but the model has provisions for a first-year survival decrement of translocatees as compared to the native born seals at the release site. The concept underlying this survival decrement is based primarily on data supporting a positive relationship between weaning girth and first year survival, although the shape of that relationship varies over time and space (Baker 2008). Weaned pups in the MHI exhibit higher survival than in the NWHI and also MHI pups wean in far better condition on average than in the NWHI. Therefore, if we were to translocate NWHI weaned pups to the MHI, we would not necessarily expect them to enjoy the average survival rate of native pups, but rather the survival rate of *similarly-sized* pups in the MHI, as predicted by the fitted relationship between size (girth) and survival in the MHI. The average girth of 70 weaned pups born at FFS during 2007-2009 was 103.7 cm. Pups in the MHI with this girth would have an expected survival rate of 0.69. The overall survival rate of pups born in the MHI is 0.77, so that the expected decrement for FFS pups translocated to the MHI would be $0.69/0.77 = 0.90$. This value was used for the survival decrement in certain translocation scenarios. To encompass the full range of

possibilities, additional scenarios were run using no survival decrement for the first year after release at the nursery site. In a review of a variety of past translocation experiences, Baker *et al.* (2011b), found that translocated weaned pups enjoyed survival rates indistinguishable from native born seals in the same area.

For all simulation years subsequent to the first year after release, translocated seals shared the same survival rate as native-born seals with survival determined stochastically as described above. However, the model maintains separate “accounting” for the translocated seals so that the number of seals stochastically surviving to each age is tracked.

The model provides the option to return seals to their natal site at a specified age. For all of the simulated translocations described herein seals were returned at age 3 yr. While some seals may in fact be returned at age 2 yr, for illustration purposes it is helpful to simulate returns at a single age. Additionally, for assessing the largest effects of two-stage translocation, it is informative to simulate the case in which all seals would be returned at age 3 yr. This scenario has the greatest lasting effect on the natal population and the greatest transient effect on the nursery population abundance. At this stage of the simulations, another survival decrement can be optionally applied to represent differential success relative to non-translocated seals left on site. As with the previous nursery site survival decrement, the return decrement applies only to the first year after release. The appropriate magnitude for this decrement is uncertain, but multiple factors might act to steer this adjustment in opposing directions. Returning seals will initially be unfamiliar with the new environment and it might take some time for them to orient to prime foraging and haulout areas. The available prey may also differ between the two areas. Returning seals may have less experience with sharks and competitors, especially if they grew up in the MHI. Finally, because there has been little experience translocating seals of this age, there may be some increased mortality due to stress of captivity. In contrast to the preceding negative considerations, and in accordance with the intent of the translocation to place seals in a more favorable environment, returning seals may be larger and healthier than seals that developed on site. This factor would positively affect survival of these seals.

Due to uncertainty regarding the relative roles that each of these factors might play in the survival prospects of returning seals, the simulations allowed for two different return decrements: no decrement (*i.e.*, same survival as native born seals), and a 29% decrement (multiplier of 0.71) relative to native seals. The latter decrement was derived from observations of the survival of seals collected at FFS for captive care treatment and later released at Kure Atoll or Midway Atoll. While those seals had a survival rate of 71% as compared to native seals, that reduction may be more severe than is expected in the current case. The captive care seals had no foraging experience prior to release, and were age 1 yr (rather than age 3 yr) when released. Nonetheless, we believe that the two values we used (100% and 71% of native survival) are reasonable estimates to bracket the range of plausible decrements that could be expected.

Combining the two values for each of the two survival decrements, and allowing for the two different geographic scenarios (FFS to MHI, and FFS to LAY), gives a total of 8 translocation scenarios plus the single baseline (no translocation) scenario (Table 2).

Table 2. Simulation scenarios to evaluate expected outcomes from two-stage monk seal translocations. All scenarios involved 10 seals translocated per year for 5 consecutive years, with all survivors returned to their natal site at age 3 yr. Populations were initialized at current age/sex status and projected forward 10 years.

Survival multipliers 1 st year after release*		Locations (natal site to nursery site)	
Nursery (recipient) site	Natal (source) site	FFS to MHI	FFS to LAY
1.0	1.0	Scenario 1a	Scenario 2a
0.90	1.0	Scenario 1b	Scenario 2b
1.0	0.71	Scenario 1c	Scenario 2c
0.90	0.71	Scenario 1d	Scenario 2d

* Values in each cell are multiplied by operative rate for like age-class seals at the release site to provide an adjusted survival rate applicable to the treated seals.

Metrics for evaluation

It is important that a proper metric, or set of metrics, be identified to evaluate the outcomes from the translocation simulations. In the long term, critical metrics include total population abundance, metapopulation structure and extinction risk. These measures clearly depend on a wide range of factors (many of which are represented in the model along with their associated uncertainties), which collectively account for the substantial variability in outcomes characteristic of long-range projections. Although conducting long-range projections, and perhaps full population viability analysis (PVA), is vitally important in the strategic design of monk seal recovery, it is not our intent to undertake such an analysis here. Rather, we are primarily interested in near-term projections and metrics that are most useful for revealing the influence of the proposed translocations, and which minimize the confounding influence of other factors (density dependence, environmental stochasticity, etc.) that might mask the direct effects of the translocations.

Among the obvious metrics for assessing results from the simulations is raw population abundance or realized growth rate from the first to final years of the simulations. While these values are certainly informative, we believe that they can be misleading because they fail to address one of the salient limitations in the NWHI subpopulations, that of a depauperate age structure. As described in the background section, the protracted period of low juvenile survival has led to an ageing breeding population and dwindling cohort sizes. Barring a natural improvement in juvenile survival, or an intervention that addresses the same, that pattern is expected to continue for the foreseeable future. Within that context, it is appropriate that the simulations be evaluated according to some metric associated with population age structure. *Reproductive value* (v_x), and the related *population reproductive value* (V_{pop}), provide informative measures for this purpose. Age-

specific reproductive value (Eqn. 1) reflects the probable future reproductive output of an individual female now of age x in terms of newborn equivalents. This value is given by:

$$v_x = \frac{\lambda^x}{l_x} \sum_{i=x}^{\max} \frac{\phi_i}{\lambda^i} \quad (1)$$

where λ is the intrinsic growth rate, l_x is the survivorship to age x , and ϕ_x is the age-specific net maternity function ($l_x m_x$).

Reproductive value is a particularly useful descriptor for comparing the relative demographic contributions expected from individuals of different ages. It incorporates information on both the likelihood of survival to each reproductive age, as well as the expected reproductive output of an individual of age x and all future ages. It is less useful for comparing across lifetables (that is, among different populations) since it is scaled in terms of newborns for the unique lifetable applicable to that particular site. For monk seal populations, v_x attains a maximum at around age 5-7, but varies in maximum value from over 7 newborn equivalents (FFS) to under 3 newborn equivalents (MHI) (Figure 6). The difference between these two sites is largely attributable to the fact that at FFS, newborn pups stand a poor chance of reaching the age of reproductive maturity, whereas the prospects for pups born at the MHI are relatively high.

Whereas v_x is a property of the lifetable and does not reference the current population state, *population reproductive value* (V_{pop}) extends the concept by incorporating information on the current population size and age/sex composition. This parameter is the sum of the age-specific reproductive values for all of the females currently in the population:

$$V_{pop} = \sum_{x=0}^{\max} v_x n_x \quad (2)$$

where v_x is the age-specific reproductive value of an individual of age x , and n_x is the number of individuals of age x currently in the population. One can think of V_{pop} as analogous to the quantity of potential energy stored in the population, which is likely to translate into future pup production. This metric is particularly *apropos* for our purposes because we do not believe that any single intervention, including translocations, will be capable of effecting a major improvement in total population abundance. We do believe, however, that by targeting our interventions on age-structure adjustments, we can fortify the population so that it is capable of a rapid response should environmental conditions more conducive to population growth eventually arise.

Using these two demographic measures as our primary metrics, what we hope to achieve through translocation is to increase the number of females in those age classes having the highest v_x . In aggregate, those additional females will act to increase V_{pop} . This concept is best illustrated graphically (Figure 7). Here we see the resulting age structure from a hypothetical translocation scenario, as compared to the baseline, no-translocation projection. The increase in number of females aged 5-9 yr corresponds to the age classes with the highest v_x at FFS (dotted line and right y -axis). By taking those seals to a more

favorable nursery site, they will effectively circumvent the intense survival bottleneck affecting non-translocated seals left on-site.

Simulation Results

Effects of the translocations at the nursery site

Because the translocated seals were returned to their natal site at age 3 yr for the simulations, the effects of the translocations at the nursery site were ephemeral (Figure 8a). As expected, final abundance at the nursery site was the same with or without the translocations, but the mean population trajectory was elevated while the project was underway (years 1-8) as compared to the baseline trajectory. This observation holds true for all 8 translocation scenarios. This pattern of no net effect is based on the assumption that the addition of a small number of seals at the nursery site (maximum of 30 at any time, age pup through age 2) will not result in density-dependent reductions in survival at the nursery site. Further, the imported seals were “removed” prior to attaining reproductive maturity and therefore produced no pups at the nursery site. Because the translocations elicited no net change at the nursery site, the remainder of this review will focus on effects at the natal site.

Effects of the translocations at the natal site

For all scenarios, the natal population (FFS) was initialized at the current (2010) population size of 194 seals. The mean abundance declined under all simulation scenarios, including both the baseline (Bsl) and all translocation scenarios. In the no-translocation scenario (Bsl Figure 9), the abundance dropped to 93 seals at the end of the 10-year projection (52% decline). The projected decline is largely driven by loss of senescent seals and a declining cohort size from fewer breeding females. Although the benefits derived from translocations were not sufficient to fully compensate for the population decline forecast for this site, the final abundance with translocation ranged from 96 to 112 seals, depending on which site was used as the nursery (MHI or LAY) and which set of survival decrements was applied. The highest abundance (112 seals) was achieved when the seals were taken to the MHI and no survival decrements were applied.

When viewed in terms of their effects on *population reproductive value* (V_{pop}), returns from the simulated translocations were more impressive. However, as with final abundance, none of the translocations were sufficient to offset the expected decline from all other factors (Figure 10). Initially (year 1) the FFS population has V_{pop} of approximately 360 newborns (this value varies each simulation due to random age assignments of seals having unknown ages, such as those first identified as adults). Under the no-translocation scenario (Bsl), the V_{pop} is expected to decline to less than 165 newborn equivalents. In contrast, under the various translocation scenarios, V_{pop} ranged from 181 to 263 newborn equivalents. As with final abundance, the greatest returns were achieved through the MHI translocation scenarios (T1a to T1d), but even the least favorable translocation scenario

(T2d; LAY with both survival decrements) produced a 10% improvement in V_{pop} as compared to the baseline scenario.

Yet another way to view the returns from the translocations is by inspecting the proportional change in V_{pop} from year 1 to year 10 of the scenarios (Figure 11). With no intervention, in 10 years the FFS subpopulation is expected to have only about 45% of the reproductive potential of the initial population. Under the most favorable translocation scenario (T1a), approximately 73% of V_{pop} is preserved, with the remaining translocation scenarios yielding between 50% and 70%.

Interpretation of Simulation Results

It is evident from the simulations that FFS is likely to undergo a significant decline in both abundance and reproductive capacity with or without focused intervention. The best that can be achieved through translocation is to moderate the decline and reinforce the population so that it has enough resilience to capitalize on improved conditions should they occur, and to initiate a slow natural recovery which might be bolstered by additional interventions. The simulations described above are all focused on a single subpopulation, FFS, which currently has the poorest juvenile survival and lowest intrinsic growth rate of any breeding site. The general pattern described for FFS, along with the expected benefits from translocation, are applicable to all of the NWHI subpopulations. The magnitude of the benefit conferred through translocation will vary according to the current status of the subpopulation and the survival differential between whichever natal and nursery site are selected for treatment, as based on the decision framework presented above.

The specifics of the 8 simulation scenarios we described were chosen to illustrate the range of benefit that might be realized from two-stage translocation. Although the specifics of these scenarios were hypothetical, it is worth considering which among them we believe to be the most realistic. For the FFS to MHI translocations (T1a – T1d), there is a reasonable expectation that the first survival decrement (0.90 multiplier for the first year after release) will apply due to the smaller size and inferior condition of FFS pups relative to MHI pups. The post-return decrement is less certain; it is likely that the 0.71 survival multiplier is overly severe, as it was based on a set of captive care seals released at age 1 yr and having no prior foraging experience. These observations lead us to conclude that the actual benefit from translocation to the MHI would be intermediate between scenarios T1b and T1d.

We can apply the same logic to the LAY translocations (T2a to T2d). First, the initial decrement is likely to be less than the 0.90 multiplier because seals born at FFS and LAY are more similar in size and condition than are seals born at FFS and MHI (as used to calculate the 0.90 decrement). Therefore the actual multiplier is expected to be less severe than that prescribed by the 0.90 value used for the MHI. Similarly, because the seals will be returned to habitat that is similar to that in which they developed (*e.g.*, in terms of predators and competitors), the returning decrement could arguably be less severe than that for seals transferred from the MHI to FFS. It is reasonable to expect that *some* decrement will be incurred as the seals orient to the new area, so that the correct value for

the second multiplier will lie between 0.71 and 1.0 but probably on the higher end of that range. This logic leads us to conclude that the most realistic scenario is a composite of scenarios T2a, T2b and T2c.

There is another very important consideration with regard to the FFS to LAY translocations and which may be applicable to any within-NWHI translocation scenario. In contrast to the MHI, each of the NWHI subpopulations is currently declining. Consequently, it is questionable whether any of these sites could accommodate additional seals without causing further depression in survival rates. Further, substantial inter-annual variability in vital rates in the NWHI may make it difficult to identify which combination of sites might reliably produce a positive outcome in a given year. This same variability could also make it difficult to discern whether any downturn in demographic performance was related to translocation efforts or attributable to normal stochastic variation. There are, however, clear advantages to within-NWHI translocations. Confining the interventions to the NWHI circumvents potential problems with human-seal interactions and public resistance to importing, even if only temporarily, additional seals. Disease and quarantine concerns might also be less intense in the context of exclusively within-NWHI translocations.

Addressing uncertainty in post-return decrements to survival

The simulated benefits of two-stage translocations are strongly influenced by the magnitude of decrements applied to survival of translocated seals after each translocation stage. The decrement values used for the simulations were extrapolated from the best available data and are a reasonable expected range based on existing information. There has been considerable experience translocating weaned pups (Baker et al.,2011) and much analysis of the relationship between weaning girth and survival (Baker 2008), so that the expected range of survival decrements applied to translocated weaned pups is well supported. However, there is much greater uncertainty associated with the decrement applied to 3-yr-old seals returned to their natal subpopulations. Given this uncertainty, it is informative to consider how large a survival penalty translocated seals could incur before their survival matched, or was inferior to, that of non-translocated seals at the natal site. This threshold decrement value can be estimated from observed survival rates for seals at the natal and nursery sites (Table 3).

Table 3. Age-specific survival rates for recent years at FFS, LAY and MHI. The rates in the first column represent survival from weaning to Age 1.

	Weaning to 1 yr	1 yr to 2 yr	2 yr to 3 yr	3 yr to 4 yr
FFS	0.359	0.567	0.941	0.895
LAY	0.681	0.537	0.917	0.938
MHI	0.841	0.859	0.910	0.891

In the above simulations, FFS served as the donor site and MHI or LAY served as the nursery sites. Seals were returned seals to their natal site at age 3 yr, at which point a survival decrement was applied for the first year after return (from age 3 to 4 yr). Therefore the value of greatest interest for evaluating translocation is survivorship from weaning to age 4, designated as l_4^* (the asterisk serves to distinguishes this parameter from the customary l_4 which measures survival from birth to age 4), which is the product of the age-specific survival rates in Table 3):

$$l_4^* = p_0 * p_1 * p_2 * p_3 \quad (3)$$

where p_0 is the survival rate from weaning to age 1 and p_1 - p_3 s are age-specific survival rates for the respective ages. Substituting the survival rates for ages 0-3 yr at FFS (Table 3) into Equation 3 gives $l_4^* = 0.171$. Accordingly the objective of the translocations is to improve on that rate such that the translocated seals do better than those “control” seals left at the natal site.

The operative survival schedule for the translocated seals is a composite of the survival rates for ages 0-2 yr at the nursery site, and age 3 yr at the return site. Additionally, we have incorporated two survival decrements that apply, respectively, to age 0 yr (weaning, when the seals are first released at the nursery site) and age 3 yr (after they are returned). The operative survival schedule for the translocated seals is then:

$$l_4^* = (p_0 * d_1) * p_1 * p_2 * (p_3 * d_2) \quad (4)$$

where p_0 , p_1 , and p_2 are the survival rates for weaning through 2 yr at the nursery site; p_3 is the survival of age 3 yr seals at the return site; d_1 is the survival decrement for pups during the first year after release, and d_2 is the survival decrement at the return site for the first year after release.

The most severe d_1 survival decrement used for the simulations was 0.90, derived from examining the survival of MHI pups of comparable girth to average FFS pups. However, because the difference in weaning girths among the NWHI subpopulations is far less than the difference between NWHI and MHI pups, a d_1 value of 0.90 may be overly severe for translocations between NWHI subpopulations. Yet, to determine survival decrement thresholds, we can conservatively set d_1 to a fixed constant = 0.90, leaving only decrement d_2 as an unknown:

$$0.171 = (p_0 * 0.90) * p_1 * p_2 * (p_3 * d_2) \quad (5)$$

where 0.171 is the aforementioned l_4^* for FFS-born, non-translocated seals. This equation serves as the basis for calculating the threshold return decrement, d_2 , that demarcates a net benefit from net harm associated with two-stage translocation.

For FFS to MHI translocations, substituting MHI survival rates for p_0 through p_2 , and the FFS rate for p_3 in Equation 5 gives:

$$0.171 = (.841 * 0.90) * 0.859 * 0.910 * (0.895 * d_2) \quad (6)$$

Solving for d_2 gives a return decrement value of 0.324. This means that, given recent survival rates at FFS and MHI, seals translocated from FFS to MHI as pups and returned at age 3 yr would do better than non-translocated seals if their realized survival for the first year after return is at least 32% that of non-translocated seals.

For FFS to LAY translocations, substituting LAY survival rates for p_0 through p_2 , and the FFS rate for p_3 gives:

$$0.171 = (.681 * 0.90) * 0.537 * 0.917 * (0.895 * d_2) \quad (\text{Eq. 7})$$

Solving for d_2 gives a return decrement value of 0.635. This means that, given recent survival rates at FFS and LAY, seals translocated from FFS to LAY as pups and returned at age 3 yr would do better than non-translocated seals if their realized survival for the first year after return is at least 63% that of non-translocated seals.

The preceding calculations of expected survival decrement thresholds are point estimates which do not account for high inter-annual variability which characterized monk seal survival, or the demographic stochasticity associated with small sample sizes (reflected in Fig. 9-11). Nonetheless, these estimates suggest that there is a sizable safety buffer for MHI translocations and a marginal safety buffer for within-NWHI translocations even if the lowest value used in the above simulations (0.71) was overly optimistic. The actual degradation in survival could be more severe than assumed and the translocated seals are still likely to perform better than seals left at their natal site.

The intent of two-stage translocation is not to merely “break even” but rather to confer enough benefits on the managed subpopulation to warrant the effort, expense and risk involved. Whether or not a particular translocation plan is advisable must still be determined according to the expected benefits (abundance, V_{pop} , and other metrics) likely to accrue from implementing that plan. However, the threshold values provide a valuable reference for maintaining a standard of “doing no harm” with the proposed program.

Under two-stage translocation, the earliest data about the actual return survival decrement would likely not be available until the fourth year of the project, when the survival of the first group of 3-yr-old seals returned to their natal sites would be evaluated. Some information could be available in the third year if some 2-yr-olds are returned. 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 seals (at least age 2 yr) 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, older juveniles 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.

Conclusion

The two-stage translocation strategy described and analyzed above is but one tool in a suite of interventions now planned or proposed to promote monk seal conservation. Unfortunately, none of these interventions, whether undertaken singly or in concert, are sufficient to fully compensate for the projected decline in the species. Although we know of no direct precedents for two-stage translocation, and there are many unknowns that accompany its implementation, we think that this approach will be indispensable to the overall recovery effort.

Two-stage translocation is a novel strategy that should produce not merely an ephemeral boost in abundance, but, more importantly, will preserve essential reproductive potential within the population. This intervention will be flexible and adaptable, with the specific form it assumes each year informed by the most recent data on demographic performance at each site. This flexibility will allow demographic issues throughout the system to be addressed, whereas some prior interventions have focused on specific mortality factors at individual sites. Those interventions are vitally important to the welfare of specific subpopulations, but they lack the scope to insulate the population from further system level decline and perhaps extinction.

The decision framework represents how the translocation program is expected to be conducted. Similarly, the simulations provide the best assessment of the returns that could be achieved through translocation. Once the program is underway, both the model inputs and details of the decision framework will be iteratively refined to reflect new observations from incoming data. Accordingly, we intend to embark on this project with the utmost caution, initially as a small-scale experiment to refine the protocols, evaluate the early results, and modify and scale up the program as appropriate.

The need to identify beneficial interventions does not end with translocation, as the NMFS will continue to identify other creative strategies to arrest the population decline. But such a solution has proven elusive, and given the current trends, it would be imprudent to defer decisive action while the quest for that ultimate remedy goes forward. It is our hope that the need for translocations, along with the need for all other intrusive measures, will eventually yield to natural processes, as the trajectory of the monk seal population begins its ascent to a sustained and full recovery. In the interim, it is incumbent on NMFS to take the steps necessary to ensure that the population is not indifferent to any improvement in natural conditions, but retains the capacity to respond accordingly.

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Figure 1. The Hawaiian Archipelago and Johnston Atoll

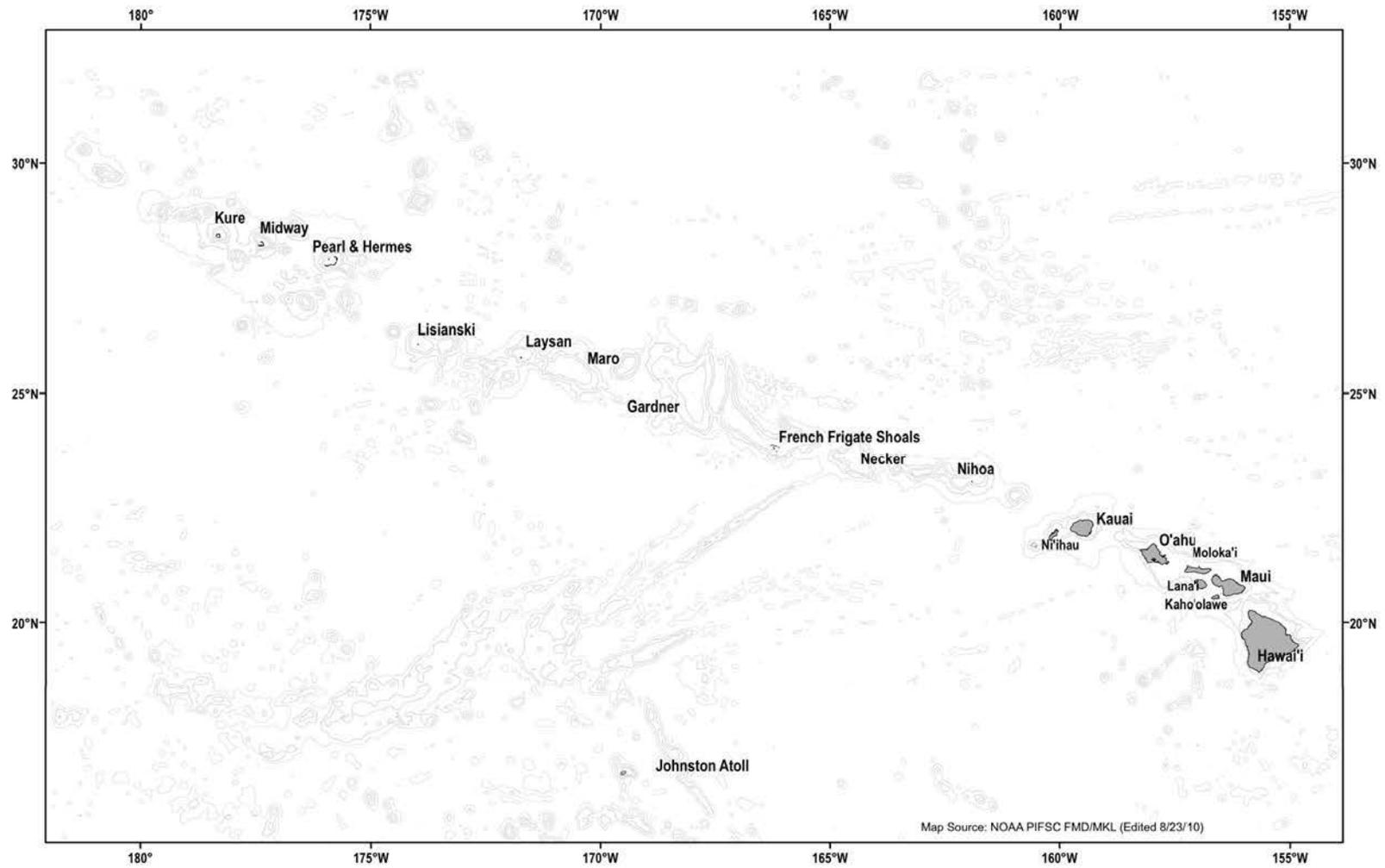


Figure 2. Cumulative survival probability curves (l_x) for the six Northwestern Hawaiian Islands subpopulations (solid lines), based upon recent (2006-2008) rates, and all available data in the main Hawaiian Islands (dashed lines). From Baker *et al.* (2011a).

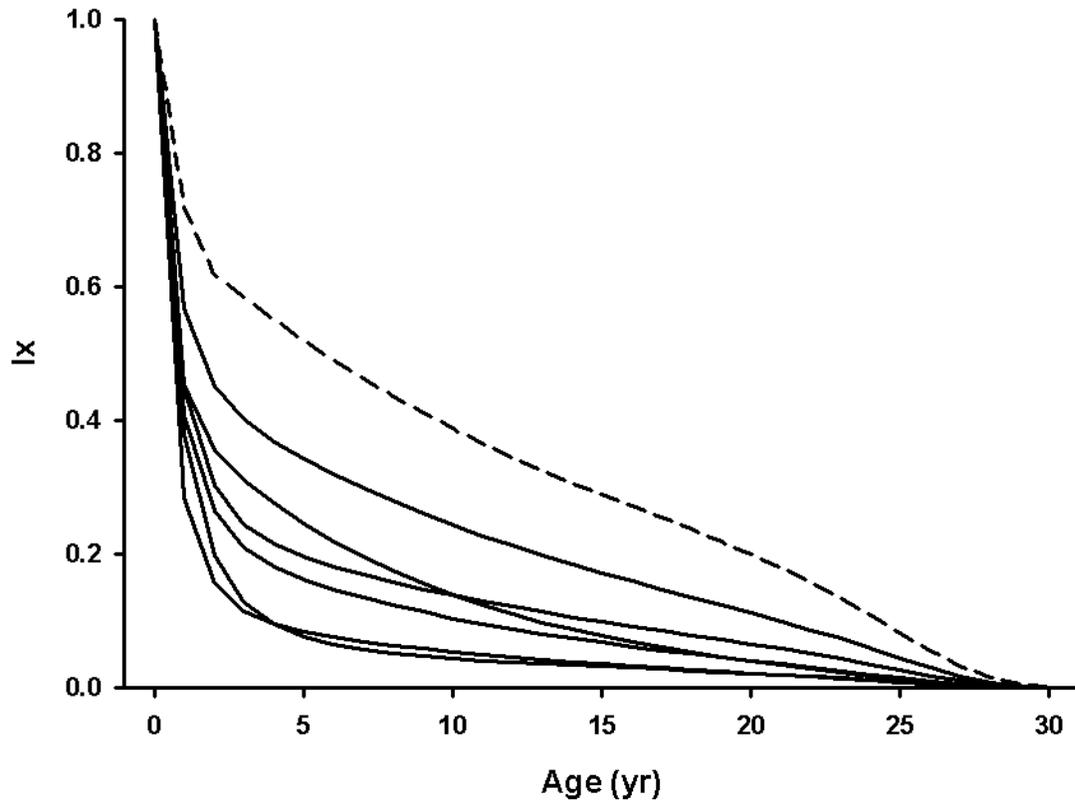


Figure 3. Fitted age-specific reproductive curves for three subpopulations of Hawaiian monk seals (LAY= Laysan Island, FFS=French Frigate Shoals, LIS=Lisianski Island).

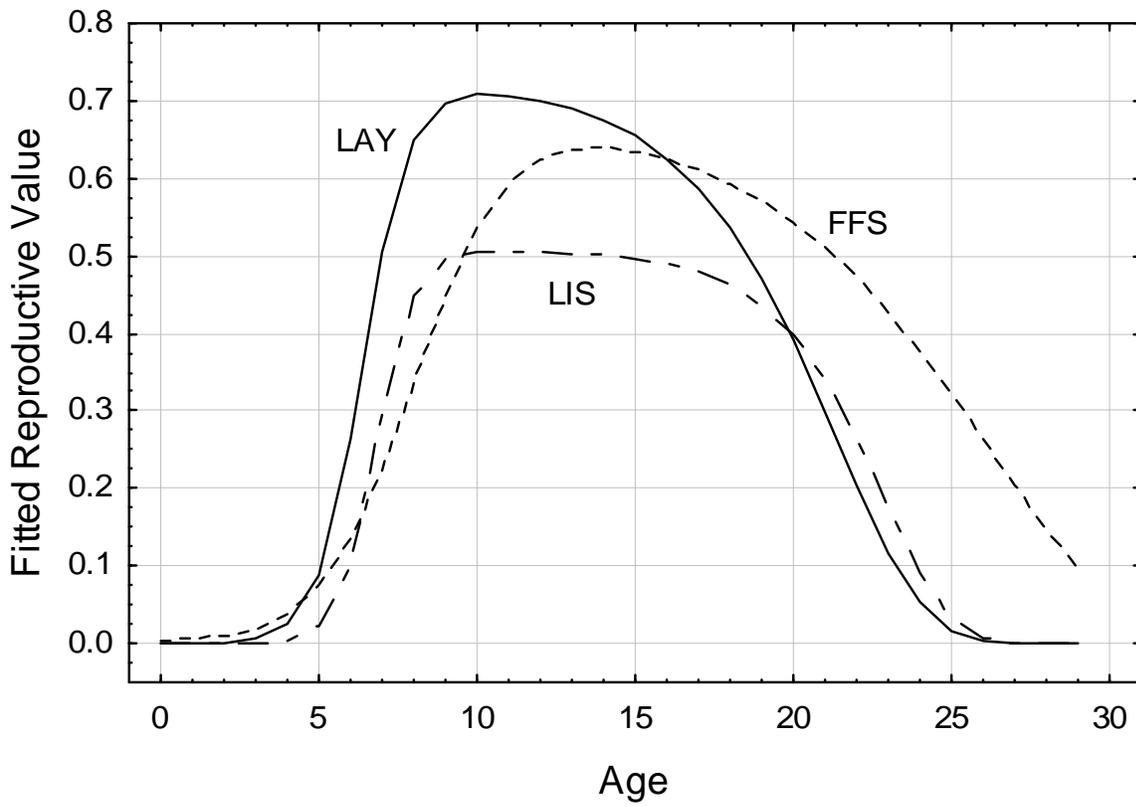


Figure 4. Simulation model projection of future Hawaiian monk seal pup production at six NWHI subpopulations pooled. Values are mean number of pups born in each simulation year in a 20-year projection.

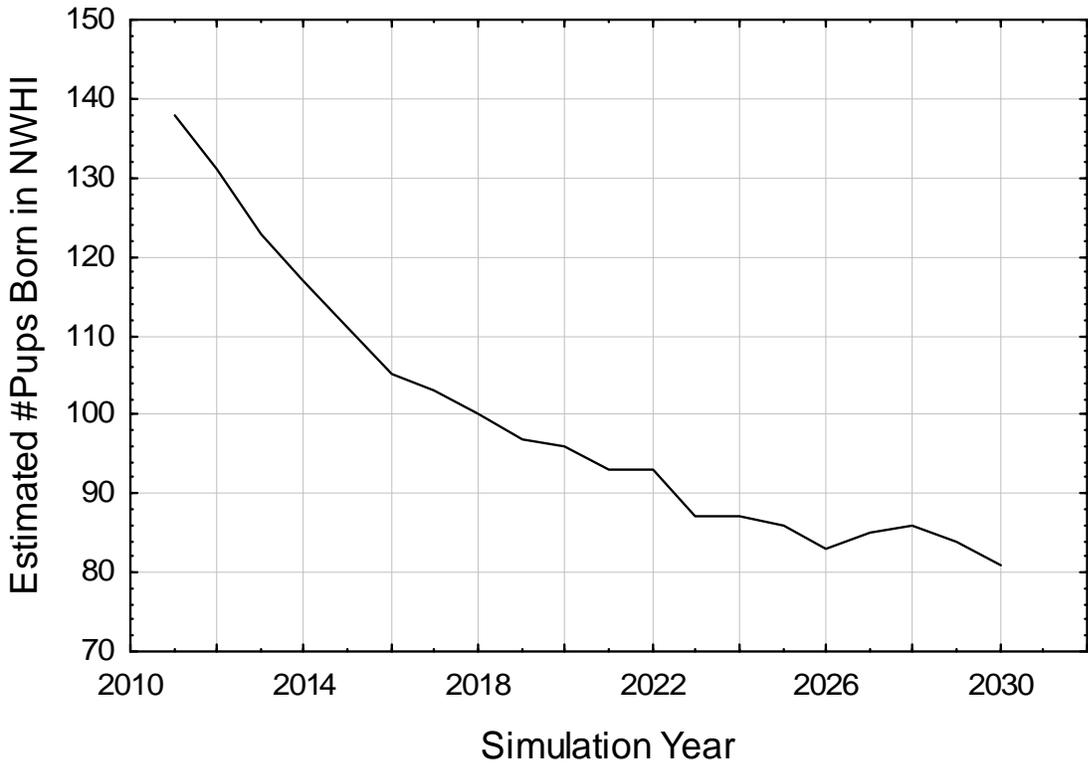


Figure 5a. Flow chart depicting decision framework for translocation of weaned Hawaiian monk seal pups.

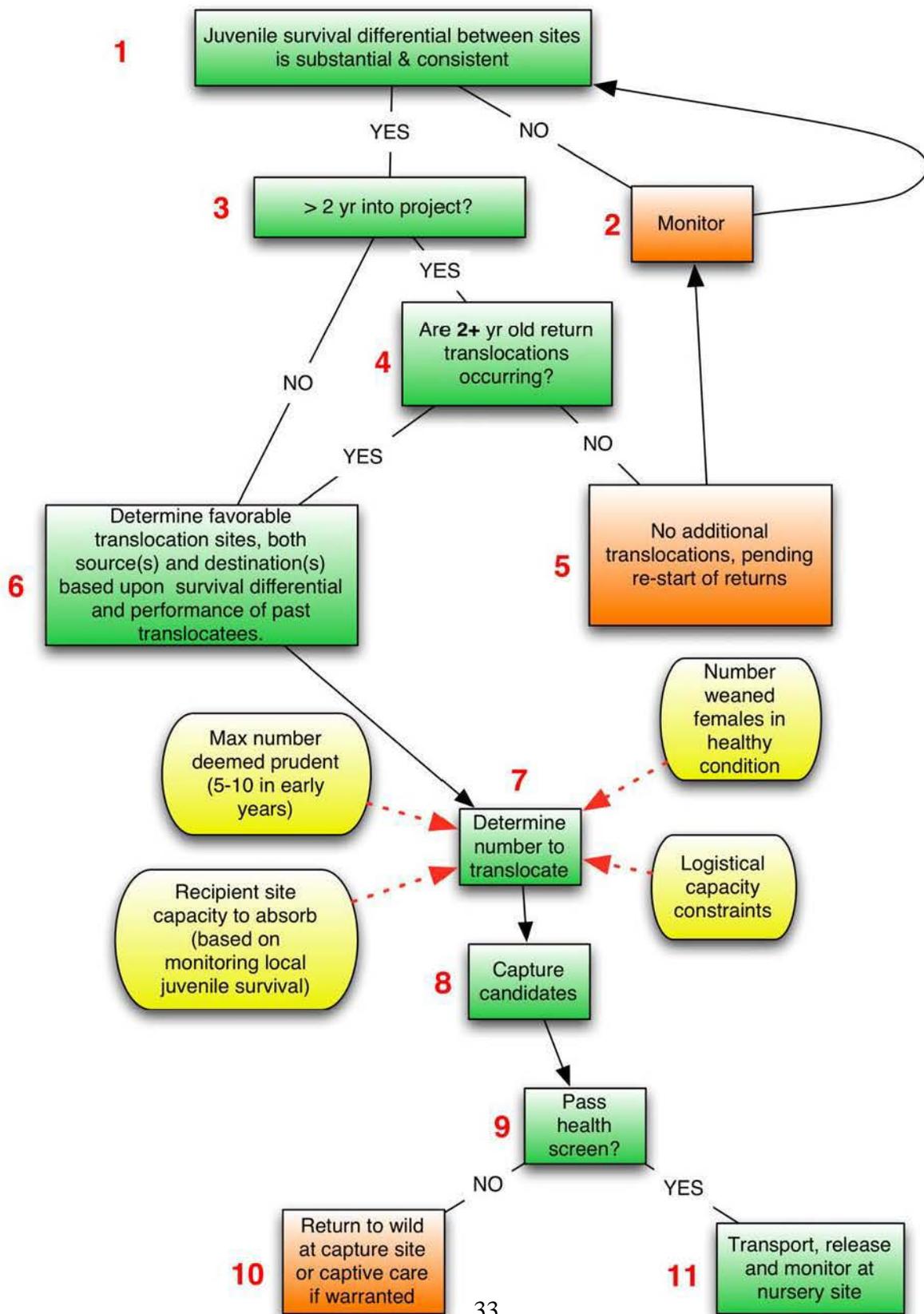


Figure 5b. Flow chart depicting decision framework for translocation of 2+ yr-old Hawaiian monk seals.

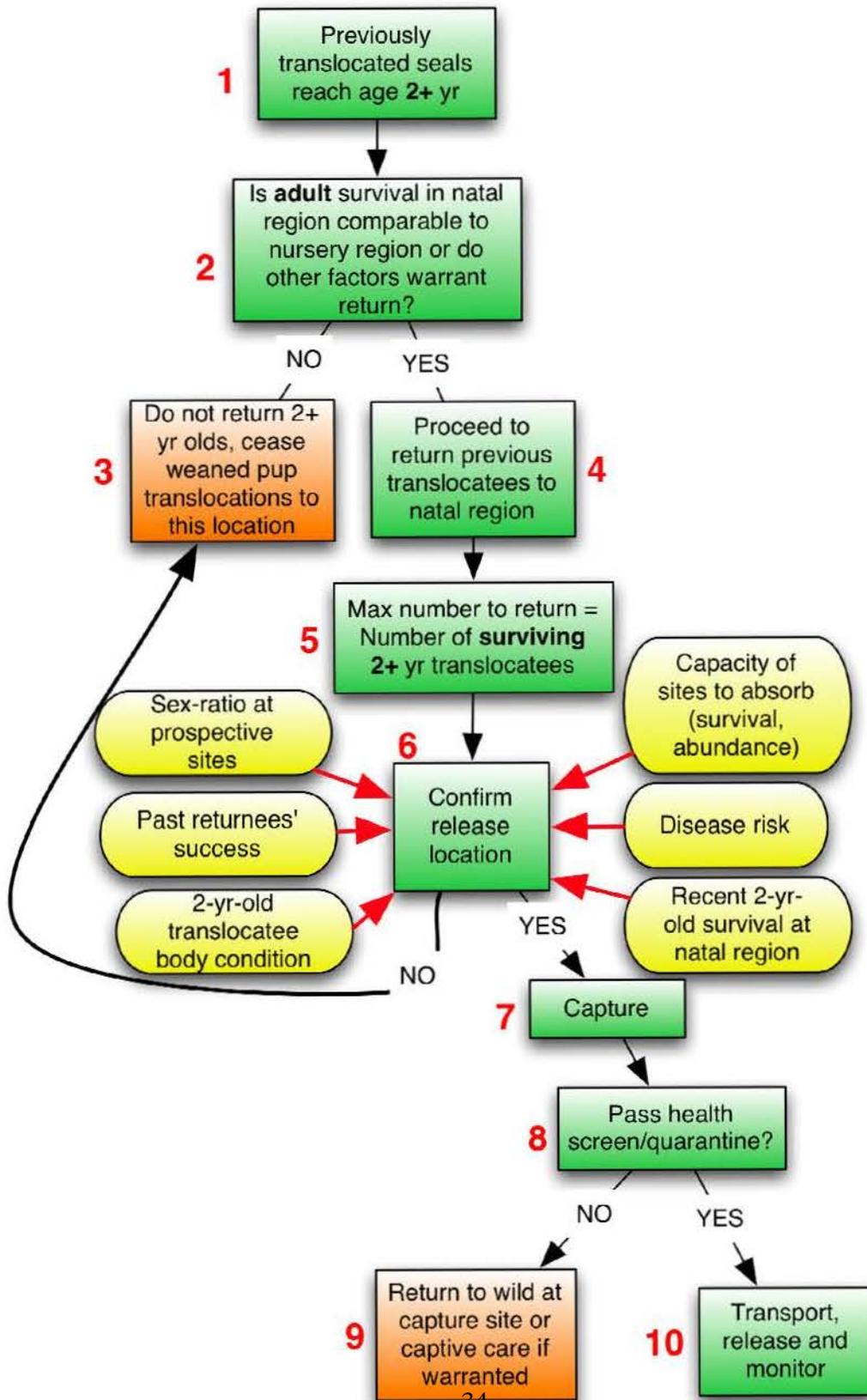


Figure 6. Contrasting age-specific reproductive value curves for French Frigate Shoals and main Hawaiian Islands MHI monk seals.

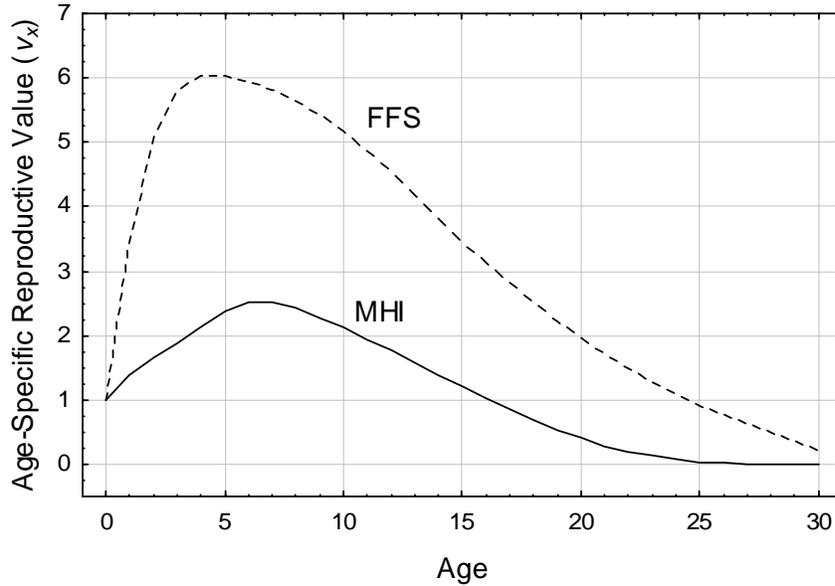


Figure 7. Age structure modification at natal site associated with a representative two-stage translocation. In this hypothetical scenario, translocated seals grow up at a nursery site and returned to the natal site at age 3, with this treatment repeated for 5 consecutive years.

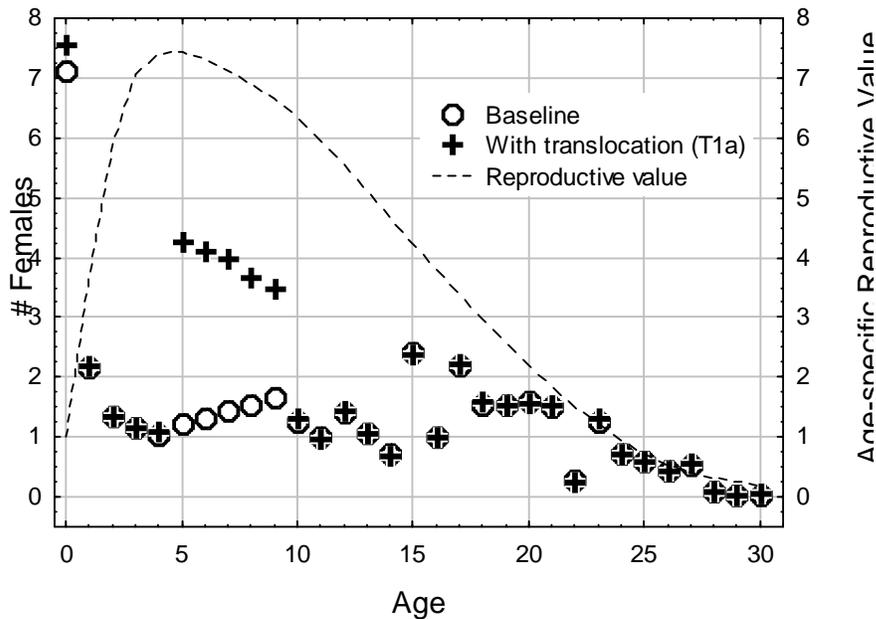
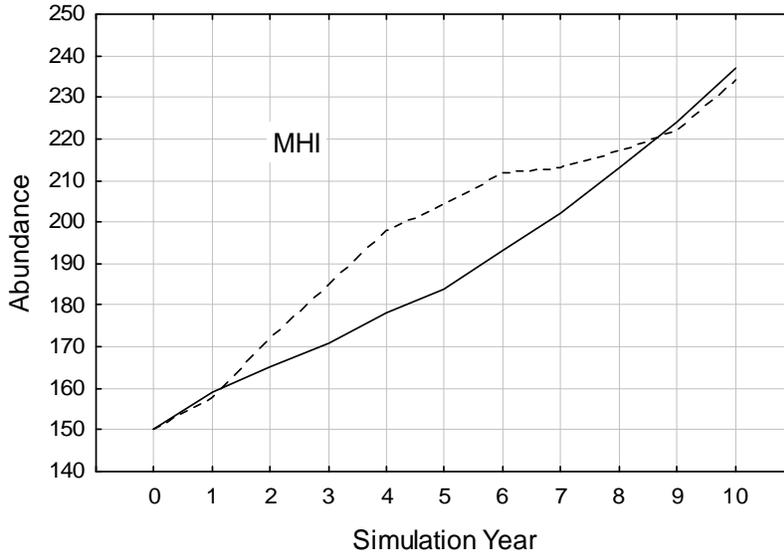


Figure 8. Simulation trajectories at the nursery (MHI) and natal (FFS) sites for a representative translocation scenario. Lines represent mean abundance at each time step, with translocation (dotted line) and without translocation (solid line). The salient difference at the nursery site is an ephemeral elevation in mean abundance during the years the project is underway.

8a. Nursery site (MHI)



8b. Natal site (FFS)

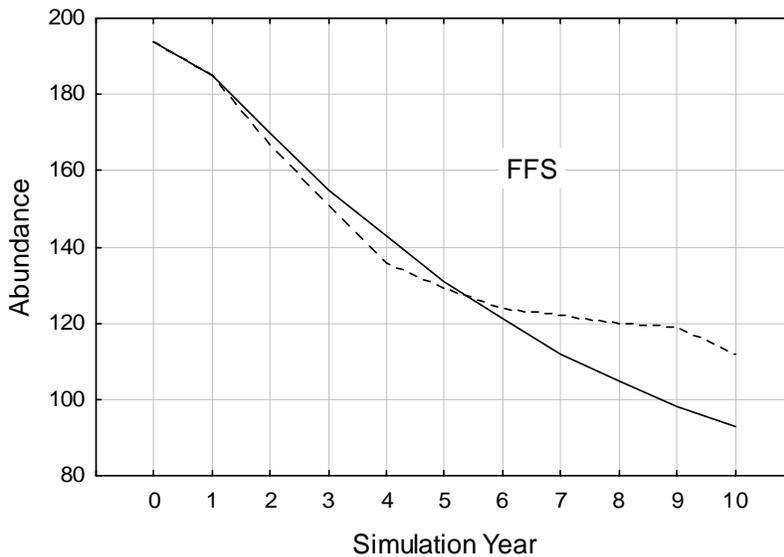


Figure 9. Mean abundance (with 5% and 95% tails) at the natal site (FFS) for the baseline (Bsl) and 8 translocation scenarios. Scenarios differ in the nursery location and survival decrements as described in Table 2.

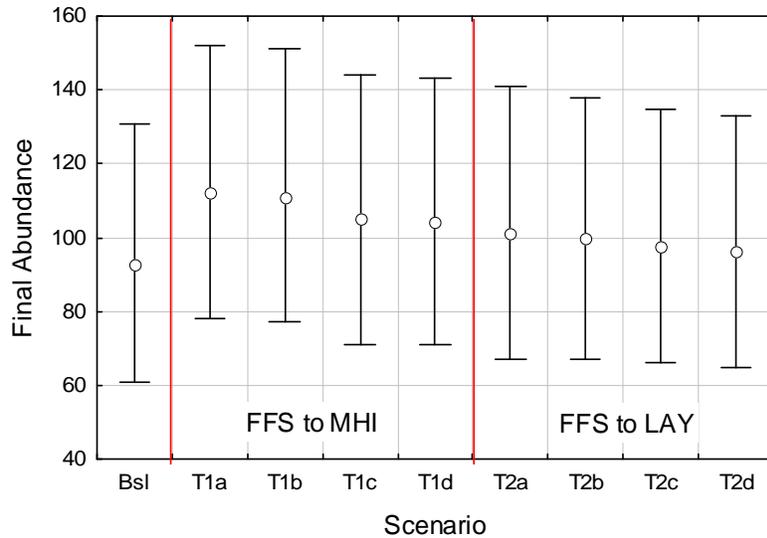


Figure 10. Population reproductive value (V_{pop} with 5% and 95% tails) at the natal site (FFS) for the baseline (Bsl) and 8 translocation scenarios. Scenarios differ in the nursery location and survival decrements as described in Table 2.

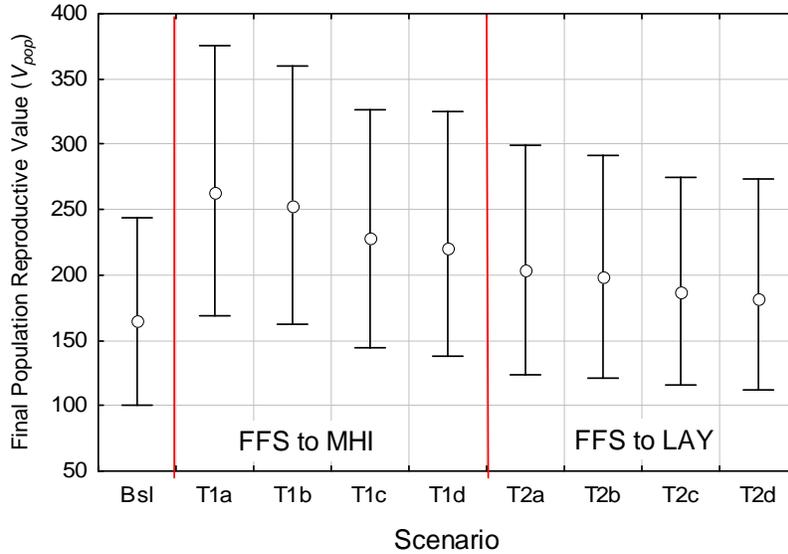
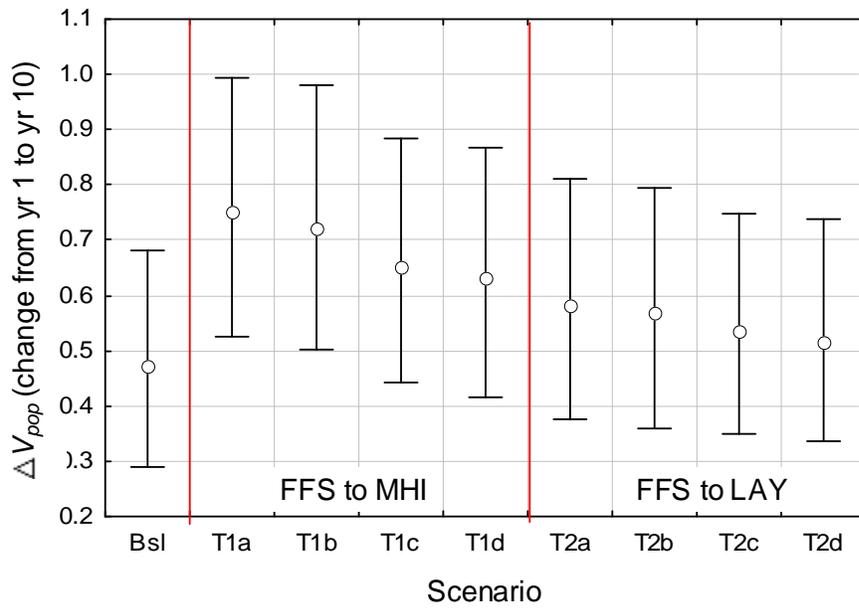


Figure 11. Change in Population Reproductive Value (ΔV_{pop}) at FFS from year 1 to year 10 of baseline and translocation simulation scenarios. Scenarios differ in the nursery location and survival decrements as described in Table 2.



File No. 16632 Appendix B: Take Tables

Table 1. Proposed annual takes of Hawaiian monk seals in the Hawaiian Archipelago and Johnston Atoll. Hawaiian Archipelago = Main Hawaiian Islands (MHI) and adjacent islets, and Northwestern Hawaiian Islands (NWHI). **MHI**=Hawaii, Maui, Molokai, Kahoolawe, Lanai, Oahu, Kauai, and Niihau. Also all smaller islands and offshore islets, including, but not limited to, Kaula Rock, Lehua, Molokini, etc. **NWHI**=Nihoa Island (Is.), Necker Is., French Frigate Shoals, Laysan Is., Lisianski Is., Pearl and Hermes Reef, Midway Atoll, Kure Atoll, Gardner Pinnacles.

Task	Size (Age)	Sex	No. Seals Taken/Year	No. Takes/Seal/Year	Type of Takes	Locations	Dates/Time Period And Details
1. Monitoring (Research)	Any	Both	250	5	Disturbance from visual observation and photo-identification during ground monitoring (including terrestrial/amphibious unmanned vehicles), vessel and aerial surveys (including unmanned aerial vehicles); and from installation and repair of remote video cameras	MHI	Annually at any time of year.
			100	3		Nihoa Is.	
			75	3		Necker Is.	
			250	5		French Frigate Shoals	
			10	1		Gardner Pinnacles	
			400	5		Laysan Is.	
			275	5		Lisianski Is.	
			400	5		Pearl and Hermes Reef	
			150	5		Midway Atoll	
			200	5		Kure Atoll	
			5	3		Johnston Atoll	

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Task	Size (Age)	Sex	No. Seals Taken/Year	No. Takes/Seal/Year	Type of Takes	Locations	Dates/Time Period And Details
2.a Tagging (Research)	Any except most nursing pups, lactating or obviously pregnant females.	Both	60	3	Restraint, tagging (flipper and PIT), collect flipper plugs, vibrissae, morphometrics (length and girth), ultrasound	MHI	Annually at any time of year (predominantly during summer field camps). Seals may also be taken by Tasks 1 and 3. Seals may also have ultrasound performed concurrent with flipper tagging At French Frigate Shoals, 35 weaned pups of either sex may have a sonic tag deployed on a third flipper tag. Any remaining nursing pups at end of field season may be tagged.
			25	3		Nihoa Is.	
			15	3		Necker Is.	
			100	3		French Frigate Shoals	
			75	3		Laysan Is.	
			70	3		Lisianski Is.	
			70	3		Pearl and Hermes Reef	
			50	3		Midway Atoll	
			50	3		Kure Atoll	
			5	3		Johnston Atoll	
			2.b Retagging (Research)	Any except most nursing pups, lactating or obviously pregnant females		Both	

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Task	Size (Age)	Sex	No. Seals Taken/Year	No. Takes/Seal/Year	Type of Takes	Locations	Dates/Time Period And Details
3. Marking (Research)	Any	Both	150	3	Temporary bleach marking	MHI	Annually at any time of year. All of the animals may also be taken by disturbance (Task 1) and tagging (Task 2).
			60	3		Nihoa Is.	
			30	3		Necker Is.	
			250	3		French Frigate Shoals	
			250	3		Laysan Is.	
			250	3		Lisianski Is.	
			250	3		Pearl and Hermes Reef	
			100	3		Midway Atoll	
			150	3		Kure Atoll	
			5	3		Johnston Atoll	
4.a Health Screening and Instrumentation (Research)	Any healthy seal excluding lactating females with pups and nursing pups	Both	100	2	Restraint, sedation, tagging, sampling (blood, swabs, blubber biopsy, vibrissae), weight, morphometrics, ultrasound, instrumentation	Hawaiian Archipelago and Johnston Atoll	Annually any time of year. Sixty (60) healthy seals may be instrumented. Recaptures for instrument removal and sampling. All animals may have been taken by Tasks 1-3.

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Task	Size (Age)	Sex	No. Seals Taken/Year	No. Takes/Seal/Year	Type of Takes	Locations	Dates/Time Period And Details
4.b Health Screening, Treatment, and Instrumentation (Research and Enhancement)	Any unhealthy seal excluding lactating females with pups and nursing pups	Both	30	2	Restraint, sedation, tagging, sampling (blood, swabs, blubber biopsy, vibrissae), bleach marking, treatment if needed (lance abscesses, administer long-acting antibiotic), weight, morphometrics, ultrasound, instrumentation, humane euthanasia or incidental mortality of 10 moribund animals	Hawaiian Archipelago and Johnston Atoll	Annually at any time of year. Includes humane euthanasia of up to 10 moribund or severely injured seals at discretion of veterinarian over a five-year period. All animals may have been taken by Tasks 1-3.
4.c Health Screening, Treatment, and Instrumentation (Enhancement)	Any unhealthy seal excluding lactating females with pups and nursing pups	Both	As warranted (est. < 30)	As directed by vet	Restraint, treatment (lance abscesses, administer long-acting antibiotic), sedation, vibrissae, bleach marking, and instrumentation	Hawaiian Archipelago and Johnston Atoll	Annually at any time of year. All animals may have been taken by Tasks 1-3. May also occur during health screening of unhealthy seals.
5.a Intestinal Parasite Treatment (De-worming Research and Enhancement)	Pups \geq 120 days post-weaning and juveniles up to age 3	Both	300	8	Restraint, weight, morphometrics, ultrasound, fecal collection (voided feces or fecal sample collected via fecal loop or digital extraction); up to 4 deworming treatments using oral or injectable drugs; up to 4 post-treatment monitoring takes at regular intervals (visual assessments and recapture for weight, morphometrics, and fecal sampling)	Hawaiian Archipelago and Johnston Atoll	Annually, year-round. Treatments may be combined with other activities requiring restraint and sedation Medical treatments authorized at discretion of consulting/attending veterinarian.

Table 1. Proposed annual takes of Hawaiian monk seals in the Hawaiian Archipelago and Johnston Atoll. Hawaiian Archipelago = Main Hawaiian Islands (MHI) and adjacent islets, and Northwestern Hawaiian Islands (NWHI). **MHI**=Hawaii, Maui, Molokai, Kahoolawe, Lanai, Oahu, Kauai, and Niihau. Also all smaller islands and offshore islets, including, but not limited to, Kaula Rock, Lehua, Molokini, etc. **NWHI**=Nihoa Island (Is.), Necker Is., French Frigate Shoals, Laysan Is., Lisianski Is., Pearl and Hermes Reef, Midway Atoll, Kure Atoll, Gardner Pinnacles.

Task	Size (Age)	Sex	No. Seals Taken/Year	No. Takes/Seal/Year	Type of Takes	Locations	Dates/Time Period And Details
				4	Restraint, weight, morphometrics, ultrasound, fecal collection (voided feces, fecal loop, or digital extraction), and topical anti-helmintic treatment		If monthly treatment determined effective during research phase, capture/restraint for follow up sampling and morphometrics would be discontinued and only topical treatment would be administered.
				8	Additional topical anti-helmintic treatments via topical application without capture and restraint (up to 12 monthly treatments annually via topical anti-helmintic);		
6.a Translocation to Save Abandoned Pups (Enhancement)	Nursing pup	Both	As warranted (est. < 20)	6	Capture, restraint, and relocation by hand to natural mother or prospective foster mother	Hawaiian Archipelago, Johnston Atoll	Establishing/re-establishing maternal association. Annually at any time of year but predominantly during summer field camps. Most takes will occur in the NWHI (intra-island/atoll).
6.b Translocation to Alleviate Risks (Enhancement)	All	Both	As warranted (est. < 60)	3	Capture, restraint, sedation, sampling (blood, swabs, blubber biopsy, vibrissae), instrumentation, temporary holding, and relocation from high risk areas via boat, ship, vehicle, or air craft	Hawaiian Archipelago, Johnston Atoll	Risk alleviation. Annually at any time of year. Translocations within or between any subpopulations in the species range allowed. Pups near weaning (e.g., within a few days of the mother leaving) and that are at high risk of mortality may be translocated. Seals may also be hazed away from dangerous locations.

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Task	Size (Age)	Sex	No. Seals Taken/Year	No. Takes/Seal/Year	Type of Takes	Locations	Dates/Time Period And Details
6.c Two-Stage Translocation (Enhancement)	Weaned Pup	Both	20	3	Capture, restraint, sedation, sampling (blood, swabs, blubber biopsy, vibrissae), instrumentation, temporary holding, translocation from areas of low survival via boat, ship, vehicle, or aircraft	Hawaiian Archipelago, Johnston Atoll	Enhance survival: 1 st stage of two-stage translocation. Annually at any time of year. Mostly females, but males when warranted. Translocations within the NWHI or from the MHI to the NWHI, are allowed, but not from the NWHI to MHI. Details to be determined through application of decision framework in Appendix A.
	Juvenile and Sub-adult	Both	30	3	Capture, restraint, sedation, sampling (blood, swabs, blubber biopsy, vibrissae), instrumentation, temporary holding, translocation via boat, ship, vehicle, or air craft Surviving juveniles that had been translocated as weaned pups returned to their natal or other suitable site (includes seals from 1 st stage of translocation that remained at recipient site until at least age 2 yr).	Hawaiian Archipelago, Johnston Atoll	Enhance survival: 2 nd stage of two-stage translocation. Annually at any time of year. Mostly females, but males when warranted. Translocations within or between any subpopulations in the species range allowed. Note that seals originally born in the MHI and previously taken to the NWHI may be returned to the MHI. Details to be determined through application of decision framework in Appendix A.

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Task	Size (Age)	Sex	No. Seals Taken/Year	No. Takes/Seal/Year	Type of Takes	Locations	Dates/Time Period And Details
6.d Translocation for Research	Juvenile, sub-adult and adult	Both	6	3	Capture, restraint, sedation, sampling (blood, swabs, blubber biopsy, vibrissae), instrumentation, temporary holding, translocate between subpopulations	Hawaiian Archipelago, Johnston Atoll	Research to determine survival of translocated juveniles to inform two-stage translocation enhancement. Annually at any time of year. Translocations within or between any subpopulations in the species range allowed. Seals with unmanageable behavior in the MHI may be translocated to the NWHI as part of this study.
7.a Adult Male Removal (Enhancement)	Adult	Male	20	2	Capture, restraint, sedation, sampling (blood, swabs, blubber biopsy, vibrissae), instrumentation/translocation, permanent captivity, or euthanasia	Hawaiian Archipelago; Johnston Atoll	Up to 20 males may be removed annually, but only 10 lethal removals over a five-year period. Taste aversion testing may occur on adult male seals brought into captivity.
7.b Adult Male Hazing (Enhancement)	Adult	Male	As warranted (est. <10)	As warranted (est. <10)	Haze	Hawaiian Archipelago; Johnston Atoll	Aggressive males may be hazed away from conspecific victims in cases of immediate risk of injury or death or when specific males repeatedly attack conspecifics.
8. Disentangle and Dehook (Enhancement)	Any	Both	As warranted (est. < 75)	As warranted	Disentanglement and dehooking (with or without capture, sedation, and release); collect vibrissae	Hawaiian Archipelago; Johnston Atoll	Annually at any time of year. All animals may have been taken by Tasks 1-3.

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Task	Size (Age)	Sex	No. Seals Taken/Year	No. Takes/Seal/Year	Type of Takes	Locations	Dates/Time Period And Details
9. Conduct Necropsies (Research)	Any	Both	As warranted	1	Necropsy any seal found dead, that died during restraint, or that was euthanized. After, use seal tissue as bait for permitted shark removals	Hawaiian Archipelago; Johnston Atoll	Annually at any time of year.
10. Opportunistic Retrieval of Samples (Research)	Any	Both	1,100	Unlimited samples	Collect parts (placentae, scats, spews, and molted fur/skin) from haul out areas	Hawaiian Archipelago; Johnston Atoll	Annually at any time of year but predominantly during summer field camps.
11. Import and Export Parts (Research)	Any	Both	Unlimited import/export	Unlimited samples	Import/export/receive	World-wide (including but not limited to Canada, the Netherlands, Scotland, Greece, Australia)	Annually at any time of year. Export (and re-import) Hawaiian monk seal samples collected under the authority of this permit. Import (and re-export) Mediterranean monk seal specimens for research related to monk seal conservation.
12. Supplemental Feeding (Enhancement)	Pup or Juvenile	Both	12	Unlimited	Supplemental feeding of post-rehabilitated seals	NWHI	Annually at any time of year seals may be fed at daily or longer intervals for up to one year.

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Task	Size (Age)	Sex	No. Seals Taken/Year	No. Takes/Seal/Year	Type of Takes	Locations	Dates/Time Period And Details
13. Behavioral Modification (Research and Enhancement)	Any	Both	20	As warranted (est. <20)	Intentional harassment for behavior modification. Aversive conditioning and other methods including but not limited to: Capture restraint, sedation, sampling (blood, swabs, blubber biopsy, vibrissae), instrumentation, translocation, temporary holding; hazing using visual, audible and tactile means; impeding movement with barriers, etc. Chemical taste aversion with lithium chloride in captivity only.	MHI	Annually at any time of year. Prevent seals from socializing with humans; alter behavior of seals socialized to humans or behaving in a manner dangerous to the seal or public safety. Seals may be brought into temporary captivity for taste aversion research. Experimental protocols to determine optimal methods.
14. Vaccinations (Research and Enhancement)	Any	Both	1,100	4	Capture, restraint, sedation, sampling (blood, swabs, blubber biopsy, vibrissae), and administration of vaccine	Hawaiian Archipelago	Annually at any time of year.
15. Incidental harassment of monk seals (Research and Enhancement)	Any	Both	400	3	Incidental harassment during any research and enhancement activity including opportunistic sample collection	Hawaiian Archipelago; Johnston Atoll	Total incidental harassment over all activities.

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Task	Size (Age)	Sex	No. Seals Taken/Year	No. Takes/Seal/Year	Type of Takes	Locations	Dates/Time Period And Details
16.a Unintentional Mortality (Research)	Any	Both	2	1	During any research activity	Hawaiian Archipelago; Johnston Atoll	Four unintentional mortalities over a five-year period not to exceed two deaths in any one year.
16.b Unintentional Mortality (Enhancement)	Weaned pup	Both	2	1	During any enhancement activity	Hawaiian Archipelago; Johnston Atoll	Four unintentional mortalities over a five-year period not to exceed two deaths in any one year.
	Juvenile/subadult	Both	4	1	During any enhancement activity	Hawaiian Archipelago; Johnston Atoll	Eight unintentional mortalities over a five-year period not to exceed four deaths in any one year.
	Adult	Male	2	1	During any enhancement activity	Hawaiian Archipelago; Johnston Atoll	Four unintentional mortalities over a five-year period not to exceed two deaths in any one year.

Table 2. Proposed annual takes of non-releasable captive Hawaiian monk seals. Activities may take place at any of the following locations in the U.S.: NOAA NMFS Ford Island Facility, Honolulu, HI; Long Marine Laboratory, University of California at Santa Cruz, CA; Sea Life Park Hawaii, Waimanalo, HI; Sea World San Antonio, TX; Waikiki Aquarium, Honolulu, HI; or any other APHIS-approved facility. Includes current non-releasable captive seals and future non-releasable seals.

Task	Size (Age)	Sex	No. Animals Taken/ Year	No. Takes/ Individual/ Year	Type of Takes	Locations	Dates/Time Period And Details
1. Chemical Behavioral Modification of Adult Males (Research)	Adult	Male	10	6	Capture, restraint, sedation, biomedical sampling, and administration of testosterone reduction agent; follow up sampling to monitor testosterone and behavior	Any facility permitted to hold adult male monk seals including but not limited to those listed above.	Annually at any time of year
2. Behavioral Modification (Research)	Any	Both	20	20	Intentional harassment for behavior modification. Aversive conditioning and other methods including but not limited to: hazing using visual, audible and tactile means; impeding movement with barriers.	Any facility permitted to hold monk seals including but not limited to those listed above	Annually at any time of year. Prevent seals from socializing with humans; alter behavior of seals socialized to humans or behaving in a manner dangerous to the seal or public safety. Experimental protocols to determine optimal methods.
3. Vaccinations (Research)	Any	Both	20	5	Vaccination on day 0 and day 14; Serum and nasal sampling for vaccine antibody study on days 0, 24, 42, 365	Any facility permitted to hold monk seals including but not limited to those listed above	Seals injected 2x/year and sampled 4x/year (first sampling combined with initial vaccine) for total of 5 takes per animal per year.

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Task	Size (Age)	Sex	No. Animals Taken/ Year	No. Takes/ Individual/ Year	Type of Takes	Locations	Dates/Time Period And Details
4. Validation Studies (Research)	Any	Both	20	As warranted	Capture, restraint, sedation, marking (flipper tagging, PIT tagging), biomedical sampling, de-worming, instrumentation	Any facility permitted to hold monk seals including but not limited to those listed above	Annually at any time of year to validate or test field methods
5. Adult Male Removal (Enhancement)	Adult	Male	10	1	Temporary holding under this permit until such time as another facility is permitted to permanently maintain the seals	Any facility meeting APHIS standards to hold monk seals	Removal of adult males to permanent captivity; temporary holding at Ford Island or other facility

Table 3. Proposed annual incidental takes of small cetaceans. Locations: Hawaiian Archipelago=Main Hawaiian Islands (MHI) and adjacent islets, and Northwestern Hawaiian Islands (NWHI). MHI=Hawaii, Maui, Molokai, Kahoolawe, Lanai, Oahu, Kauai, and Niihau. Also all smaller islands and offshore islets, including, but not limited to, Kaula Rock, Lehua, Molokini, etc. NWHI=Nihoa Island (Is.), Necker Is., French Frigate Shoals, Laysan Is., Lisianski Is., Pearl and Hermes Reef, Midway Atoll, Kure Atoll, Gardner Pinnacles.

Task	Size (Age)	Sex	No. Animals Taken/Year	No. Takes/Individual/Year	Type of Takes	Locations	Dates/Time Period And Details
1. Incidental harassment of spinner dolphins	Any	Both	500	5	Disturbance from vessel surveys of monk seals and transiting lagoons between monk seal ground surveys	Hawaiian Archipelago	Annually at any time of year.
2. Incidental harassment of bottlenose dolphins	Any	Both	20	1	Disturbance from vessel surveys of monk seals and transiting lagoons between monk seal ground surveys	Hawaiian Archipelago	Annually at any time of year.

File No. 16632 Appendix C: Hawaiian monk seal Drugs for Use in Field

Drug Name	Dosage/Route of Administration	Use in Hawaiian monk seals	Possible Adverse Effects	Pharmacokinetics
Atropine Sulfate	0.02 -0.2 mg/kg IM, IV, SC (CRC Handbook)	To treat bradycardia (slowed heart rate) or cardiac arrest; may be used as a pre-anesthetic to reduce respiratory secretions and block vagal mediated dive reflex.	<p>Generally dose related; mild effects in healthy patients; severe effects with high or toxic doses include gastrointestinal (constipation, vomiting), central nervous system (CNS).</p> <p>Benzodiazepines may potentiate adverse effects (Veterinary Drug Handbook, 4th Ed., Plumb)</p> <p>Used on numerous occasions in Hawaiian monk seals with no adverse reactions reported (NMFS unpubl. data). Used extensively in other pinnipeds during anesthesia with no observed side effects (Haulena and Heath 2001)</p>	Well absorbed with peak effects on heart rate within 3-4 minutes; metabolized in liver and 30-50% of dose excreted unchanged in urine. Half-life (the time required for the concentration of the drug to reach half of its original value) in humans is 2-3 hours.
Ceftiofur crystalline free acid	6.6 mg/kg IM (Meegan et al. 2010)	Long-acting cephalosporin antibiotic for prophylactic treatment of injuries and treatment of infections.	<p>Usually not serious and low occurrence; mild transient pain and possibility of abscess at injection site; diarrhea; hypersensitivity reactions include rash, fever, or anaphylaxis.</p> <p>Used in Hawaiian monk seals with no adverse effects (Permit No. 10137-07, NMFS, unpubl. data). No adverse reactions reported after use in humpback whales, California sea lions, northern elephant seals, and harbor seals (Gulland pers. comm.).</p>	<p>Half-life in cattle is 8-12 hours with peak levels after 30-45 minutes of intramuscular (IM) injection.</p> <p>A study at The Marine Mammal Center (Sausalito, CA) on 10 California sea lions resulted in maximum plasma concentrations at 24 hours post-IM injection; plasma drug levels at lower levels would likely be maintained for 5-8 days post-injection (Meegan et al. 2010).</p>

Drug Name	Dosage/Route of Administration	Use in Hawaiian monk seals	Possible Adverse Effects	Pharmacokinetics
Dexamethasone	0.2 - 1 mg/kg (CRC Handbook)	A glucocorticoid used for treatment of shock; may be used to treat adrenal insufficiency, inflammation, and other maladies.	Usually associated with long-term administration and manifested as clinical signs of hyperadrenocorticism; can retard growth in young animals; when given short-term, unlikely to cause significant harmful effects, even in massive doses. Few instances of use in Hawaiian monk seals with no adverse reactions reported (NMFS unpubl. data).	Half-life in dogs is 2-5 hours; biologic activity can persist for \geq 48 hours.
Diazepam	0.1-0.25 mg/kg IV	A benzodiazepine used as a sedative (anxiolytic, muscle relaxant, hypnotic) for capture events; may be used as an appetite stimulant or anti-convulsant.	Dogs may exhibit CNS excitement; in horses may cause muscle weakness and ataxia; in cats may cause irritability, depression, aberrant demeanor. Routinely used sedative in Hawaiian monk seals with no adverse reactions reported (NMFS unpubl. data).	Highly lipid soluble and widely distributed throughout the body; readily crosses blood-brain barrier and is highly bound to plasma proteins; metabolized in liver to active metabolites nordiazepam, temazepam, and oxazepam, which are eliminated primarily in urine.
Doxapram HCL	2-5 mg/kg IV (CRC Handbook) Administered at dosage of 5 ml (pups/juveniles) and 10 ml (subadults/adults)	A CNS/respiratory stimulant used to treat respiratory arrest; may also be administered during/after anesthesia.	Hypertension, arrhythmias, seizures, and hyperventilation, which are most probable with repeated or high doses. Increases myocardial oxygen demand and reduces cerebral blood flow. Few instances of use in Hawaiian monk seals with no adverse reactions recorded (NMFS unpubl. data).	After intravenous (IV) injection, onset of effect in humans and animals within 2 minutes; in dogs, rapidly metabolized and excreted as metabolites in urine within 24-48 hours after administration. Serum half-life in dogs is 2.5-3.2 hours and in humans is 20-50 hours.
Emodepside + Praziquantel	0.113ml/kg topical	Topical antiparasitic (nematocide + cetocide) used to treat intestinal roundworms and tapeworms.	Most common side effects in cats include skin and gastrointestinal reactions. Two instances of use in captive Hawaiian monk seals with no adverse effects noted; used in in wild seals under Permit No.	In cats: rapidly absorbed through skin and into systemic circulation after dermal administration; serum concentrations detectable for praziquantel after 1 hour (peak

Drug Name	Dosage/Route of Administration	Use in Hawaiian monk seals	Possible Adverse Effects	Pharmacokinetics
			10137-07 with no adverse reactions noted.	at 6 hours) and for emodepside after 2 hours (peak at 2 days); detectable for up to 28 days following administration.
Epinephrine	0.05-0.2 mg/kg IV, IM, SC, pericardial, intratracheal	Treatment for cardiac arrest with resuscitation; may also be used to treat anaphylaxis.	Anxiety, tremors, excitability, vomiting, hypertension (with overdose), arrhythmias, high levels of uric acid in blood, and lactic acidosis (with prolonged use or overdosage). Few instances of use in Hawaiian monk seals with no adverse reactions reported (NMFS unpubl. data).	Well absorbed following IM or subcutaneous (SC) injection; onset of action following SC injection is 5-10 minutes; immediate action following IV injection; does not cross blood-brain barrier; actions end by uptake into sympathetic nerve endings; metabolism in liver and other tissues to inactive metabolites.
Fenbendazole	11mg/kg twice (CRC Handbook)	An antiparasitic agent for treating intestinal roundworms.	Generally no adverse effects at normal doses; hypersensitivity secondary to antigen release by dying parasites may occur, especially with high doses; vomiting reported infrequently in dogs and cats ; well tolerated at doses up to 100x recommended. Used in research field trial in Hawaiian monk seals and in captive care; no adverse effects reported from use but difficult to administer orally in field setting (NMFS Permit No. 10137 Hawaiian Monk Seal Deworming Project: Year One Summary).	Marginally absorbed after oral administration; metabolized to active compound oxfendazole and sulfone; in sheep, cattle, and pigs, 44-50% of a dose is excreted unchanged in feces, and <1% in urine.
Flumazenil	0.05-0.1 mg/kg Flumazenil would be administered IV at a dosage of 2.5 ml (pups/juveniles)	A benzodiazepine antagonist used to reverse effects of sedative overdose (diazepam or	In humans, injection site reactions, vomiting, cutaneous vasodilatation, vertigo, ataxia, and blurred vision; deaths have been associated with its use in humans having serious underlying diseases; large IV overdoses have	Administered with rapid IV injection with therapeutic effects within 1-2 minutes; rapidly distributed and metabolized in liver; half-life in

Drug Name	Dosage/Route of Administration	Use in Hawaiian monk seals	Possible Adverse Effects	Pharmacokinetics
	and 5.0 ml (subadults/adults), repeated if necessary	midazolam).	rarely caused symptoms in otherwise healthy humans. Used in Hawaiian monk seals with no adverse reactions reported; trials with captive monk seals proved effective in reversing effects of midazolam (NMFS unpubl. data).	humans is approximately 1 hour.
Furosemide	2-5 mg/kg (CRC Handbook)	A diuretic used to treat congestive heart failure or pulmonary edema.	May induce fluid and electrolyte imbalances; reported to cause hearing loss in cats and dogs given high IV doses; other effects include gastrointestinal problems, anemia, weakness, restlessness. Few instances of use in Hawaiian monk seals with no adverse reactions reported (NMFS unpubl. data).	In dogs, the elimination half-life is approximately 1-1.5 hours; in humans, the diuretic effect takes place within 5 minutes and peak effects 30 minutes after IV injection.
Ivermectin	200 microgram/kg	An antiparasitic agent for treating intestinal roundworms; used as a heartworm preventative in captive monk seals.	Species-specific adverse effects generally from dying microfilaria or other larva, for example, swelling and itching in horses, shock-like reactions in dogs, and paralysis and staggering in cattle; may cause neurologic toxicity in mice and rats with doses slightly more than prescribed; may cause death, lethargy, or anorexia in birds. Used in captive care of Hawaiian monk seals to treat intestinal worms and used routinely on permanently captive monk seals with no adverse reactions reported (NMFS unpubl. data; Annual Report for Permit No. 455-1760).	Oral doses absorbed up to 95%; greater bioavailability after SC administration but more rapidly absorbed after oral administration; well distributed to most tissues except in cerebrospinal fluid thus reducing its toxicity; metabolized in liver and primarily excreted in feces; less than 5% is excreted in urine; elimination half-life for dogs is 2 days.
Lidocaine HCL	1-3 ml 2 % topically	A local anesthetic used to reduce pain from skin incisions	At usual doses, serious adverse reactions are rare; most common are dose-related and rare, including CNS reactions, transient nausea	Lidocaine has a high affinity for fat and adipose tissue and is bound to plasma proteins;

Drug Name	Dosage/Route of Administration	Use in Hawaiian monk seals	Possible Adverse Effects	Pharmacokinetics
		such as blubber biopsies.	and vomiting, and cardiac effects. Routinely used in Hawaiian monk seals during biopsy sampling with no adverse reactions reported (NMFS unpubl. data).	rapidly metabolized in liver to active metabolites; less than 10% of an injected dose is excreted unchanged in urine.
Midazolam	0.1-0.15 mg/kg IV, IM	An injectable benzodiazepine used as a sedative for capture events or as a preanesthetic.	Few adverse effects have been reported in humans including effects on respiratory and cardiac rates and blood pressure; other effects reported in humans include pain on injection, local irritation, headache, nausea, vomiting, and hiccups. Possibility of respiratory depression is principal concern in veterinary patients. Used in captive Hawaiian monk seals with no adverse reactions reported; trials with captive monk seals indicated midazolam safe and effective (NMFS unpubl. data; Annual report for Permit No. 455-1760).	Rapidly and nearly completely absorbed after IM injection; highly protein-bound and rapidly crosses the blood-brain barrier; metabolized in liver; elimination half-life in dogs averages 77 minutes and in humans is approximately 2 hours.
Praziquantel	10 mg/kg (CRC Handbook)	An anticestodal antiparasitic used to treat intestinal tape worms.	In dogs, oral dosing can cause anorexia, vomiting, lethargy, or diarrhea but incidence is less than 5%; greater incidences from injectable in dogs including pain at injection site, vomiting, drowsiness, and staggering gate. Used in research field trial (oral and IM) and in captive care (oral) of Hawaiian monk seals; no adverse effects reported from oral use in captive care; difficult to administer orally in field setting; swellings resulted from IM injections in field use (NMFS unpubl. data; Gobush et al. 2011).	Rapidly and nearly completely absorbed after oral administration; peak serum levels in dogs between 30-120 minutes; distributed throughout the body, crossing intestinal wall and blood-brain barrier into CNS; metabolized in liver and excreted primarily in urine; elimination half-life in dogs is 3 hours.

Drug Name	Dosage/Route of Administration	Use in Hawaiian monk seals	Possible Adverse Effects	Pharmacokinetics
Prednisolone sodium succinate	1 mg/kg	A glucocorticoid used for treatment of shock; may be used to treat adrenal insufficiency and other maladies.	Usually associated with long-term administration and manifested as clinical signs of hyperadrenocorticism; can retard growth in young animals; when given short-term, unlikely to cause significant harmful effects, even in massive doses. Few instances of use in Hawaiian monk seals with no adverse reactions reported (NMFS unpubl. data).	Biologic half-life is 12-36 hours.

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File No. 16632 Appendix D: Protocol for Field Abscess Treatment and Antibiotic Administration in Hawaiian monk seals

**NO INTERVENTION SHALL BE ATTEMPTED WITHOUT
VETERINARY APPROVAL AND
PERMISSION PRIOR TO EACH AND EVERY ATTEMPT**

Abscesses are relatively common in Hawaiian monk seals, particularly weaned pups. They often arise secondarily to bite wounds from interactions with other seals. Abscesses can grow to the size of basketballs, thus impeding daily function, and occasionally cause systemic illness (septicemia, a bacterial infection of the blood) and death. Intervention in the field is aimed at reducing the risk of septicemia and includes monitoring, careful documentation, and with veterinary approval, lancing abscesses and/or administering antibiotics. The following document provides written guidance for field responders to intervene when hands-on veterinary assistance is not available, though no attempts should be made without veterinary consultation and approval.

VETERINARY APPROVAL PROCESS

Field responders should be prepared to provide veterinary staff with the following information (at a minimum) **before** any intervention:

1. Age, size, sex
2. General nutritional status (fat, average, thin)
3. Molting status
4. Animal behavior:
 - a. When last observed?
 - b. Alert/responsive or lethargic?
 - c. Coordinated movements and good mobility?
 - d. Thermoregulatory behavior?
 - e. Any other abnormalities?
 - f. Size, shape and location of injuries
5. PHOTOS (both close-up photos of injuries as well as full body images)
6. Name of on-site team leader (must be state or federal employee)
7. Number of people able to assist in intervention attempt, should one be requested by the veterinarian

PRIORITIZED LIST OF CONSIDERATIONS

1. **Risks to human health and safety are to be minimized.** The size, health and abilities of a restraint team relative to the size and behavior of the animal will be of paramount importance in deciding whether or not to attempt intervention.
2. **Animal behavior:** The need for intervention should be determined by the animal's behavior and mentation and should not be based solely on the size of an abscess or wound.
3. **Abscess maturity:** Photographs and history will be used to determine the maturity of an

abscess and subsequent likelihood of successful draining.

- Treatment may not be indicated in many cases.
- Lancing and intervention may be used without antibiotic administration.

ANTIBIOTIC ADMINISTRATION

Use of antibiotics, type, dose and route will be determined ONLY by a veterinarian. The drug of choice that will be supplied to all islands is Ceftiofur (brand name: Excede).

Only State or Federal employees are authorized to administer antibiotic medications to wild monk seals (via pole syringe or otherwise), and only after consulting with a veterinarian. Administration of antibiotics to wild seals is an action that at this time is covered only under the Marine Mammal Health and Stranding Response permit (not under the PIFSC permit).

Antibiotics:

Ceftiofur (Excede) 200mg/ml

- 3rd generation cephalosporin (broad-spectrum, gram-positive and gram-negative bactericidal antibiotic)
- Route: Intramuscular (IM)
- Adverse effects are uncommon but potentially could include: pain at injection site, discoloration of the skin, hypersensitivity reactions.
- Long-acting (5 days)
- Dosage (6.6mg/kg) will be calculated by consulting veterinarian and instructions will be given to team leader.

WHAT IS AN ABSCESS?

An abscess is the body's way of walling off an infected region of tissue. In young monk seals, abscesses often arise from bite wounds or other trauma. Abscesses can occur in the skin, blubber, muscle, and lymph nodes and can thus range from being near the skin to deep within the tissues. Abscesses can be very large but may also have pockets, which sometimes makes finding the right spot for lancing difficult. Many abscesses are soft, but some are firm and cannot be expressed as easily. The material inside an abscess can be watery, bloody, milky, greenish, thick and even crumbly.

ABSCESS INTERVENTION PROTOCOL

All persons coming into direct contact with the seal or collecting specimens must be in good health and **wear gloves and protective eyewear**. Follow these instructions only after the veterinary team has given permission to intervene. **Interventions (including failed attempts) should be kept within 10 minutes to the greatest extent possible and should be done during the coolest hours of the day.**

1. SUPPLIES:

- Gloves
- Protective eyewear
- Scalpel blade (#10)
- Scalpel handle

12, 20 or 30 ml syringes (luer lock and curved tips preferred if available)
60 ml syringes
18g x 1.5" needle
16g x1.5" needle
Surgical scissors or forceps
Duct tape
Pole syringe with sterile hypodermic needles
Antibiotics if applicable
Betadine or 3% hydrogen peroxide for flushing abscesses after lancing

2. **Human safety comes first.** Interventions should only be performed by an experienced team and on an animal that can be safely restrained.
3. **Animal safety comes next.** Ensure that conditions are appropriate prior to attempting an intervention, and quickly abort a handling if the seal exhibits signs of stress. Please review the *Hawaiian Monk Seal Clean Protocol and Handling Protocol* (below) prior to attempting a seal capture.
4. **Restrain** the animal and feel the abscess to determine if fluid is present.
 - a. If you can't tell, insert a syringe and sterile needle into the softest part of the abscess (or spot that looks like it might rupture) and try to draw out fluid. Sometimes, the skin overlying the part of the abscess that is closest to the surface is discolored and flaky.
 - b. If no pus extracted in 2 attempts or if seal shows signs of stress, **stop here.**
5. **If fluid is present, lance the abscess.**
 - a. Ensure that animal is adequately and safely restrained.
 - i. If restraint is inadequate or becomes unsafe (for humans and the seal), **stop here.** Discuss mounting scalpel on a pole with veterinarian (see #7, below).
 - b. Ensure that the stress to the animal is within appropriate limits for continued restraint.
 - c. Uncover the scalpel blade, keeping the cutting edge away from the animal's body.
 - d. Insert blade quickly and firmly into the softest and most ventral (dependent) region of the abscess and cut deep through the skin, keeping blade oriented facing out and moving away from the animal's body. The location of the incision should be dependent enough so that the abscess will continue to drain by gravity alone.
 - e. Make two incisions, forming an X-shape to facilitate adequate drainage.
 - f. Generally speaking, incisions 1.5 to 2" long would be the *minimum* length for adequate drainage of any large abscesses.
 - g. If drainage is slow or limited, put scissor points or forceps into hole and open them to widen the aperture of the hole. If you find more an additional squishy spot, you may incise in a second location, provided that the seal and personnel remain safe.

6. **Drain abscess:** After cutting, compress the abscess to express as much fluid as possible. There may be small chunks of tissue, clots or other debris that may need to be pushed out in order to fully express the pus. Fluid may also be bloody. Use caution to prevent fluid from contacting the eyes of the seal and responders.
7. **Flush abscess:** Use a large (preferably 60 ml) syringe filled with dilute betadine or 3% hydrogen peroxide. Insert syringe tip into the incision, close skin around the tip and gently fill abscess “pocket” with fluid, then remove syringe and express it out the incision. Repeat a second time if seal continues to be restrained safely and is not too stressed (see “Seal Handling” section 6.a and 6.b. below).
8. **Lancing abscesses without restraint:** If human and seal safety deems restraint inappropriate, consider mounting scalpel blade on a pole. **Pole lancing of abscesses is a treatment that should only be attempted after conferring with a qualified veterinarian.**
 - a. Pole should be of sufficient length to facilitate escape (ex: tent pole, marine debris, bamboo)
 - b. Attach scalpel blade (#12 curved is preferred, or use #11 or 22) to scalpel handle and duct tape to pole, ensuring that no more than 1 to 1.5” of the blade extend beyond the end of the pole.
 - c. Animal should be in a location that is safe for both humans and the seal (flat, sandy beach without rocks or obstacles).
 - d. Only the person handling the pole should approach the seal.
 - e. To lance the abscess, keep the cutting edge of the blade facing out and away from the seal’s body. Make a single, directed jab into the most fluid (“jiggly”) portion of the abscess. Do not drive the scalpel deep into the muscle. Within the jiggly portion of the abscess, make your incision at the ventral-most aspect so that fluid will continue to drain after treatment.
9. **Methods of injection:** To be dictated by veterinarian. Double check all labels before drawing up drugs. Make sure not to contaminate needles or the top of the antibiotic vial.
 - a. Hand syringe - Draw up designated amount of antibiotic using a 10-20 ml syringe and 18g or 16g x 1.5” sterile needle. The larger the syringe and the larger the needle, the faster the drug will go into the animal – this is especially important to consider in cases of minimal restraint. Responders should be trained to use appropriate restraint or cross-boarding techniques. Administer antibiotic to seal’s right gluteal region if possible. Record location of injection for the Monitoring Report.
 - b. SafeTFlex pole syringe - Draw up specified volume of antibiotic in a 6-20 ml syringe as accurately as possible using a sterile needle (18-20g needle of any length). Attach a sterile needle of appropriate size (16-18g, 2-3.5” long, depending on age class and body condition) to the pole syringe. Pull the pole syringe plunger back to make room in the pole syringe for the drug volume you

just drew up. Inject the antibiotic into the pole syringe by placing the smaller needle on the first syringe into the 16-18g needle and expelling the drug from the first syringe into the pole syringe. The drugs are administered through the pole syringe by the force used to push the syringe into the right dorsal posterior end of the seal**. If possible, wait until the seal is sleeping and sneak up behind the seal to give the injection in the right gluteal region. Follow through with a forward motion until you are certain the full dose of antibiotics is given. Let the motion of the seal as it moves away from you withdraw the needle.

**This statement applies only to the SafeTFlex pole syringes. The Dan Inject Jab-Stick pole syringe is spring loaded. Refer to the step-by-step instructions in the Jab-stick carrying case for operation. JAB-STICK SHOULD ONLY BE USED BY RESPONDERS SPECIFICALLY TRAINED IN ITS USE.

POST-TREATMENT MONITORING REPORT

Seal should be observed for 30-60 minutes post-treatment/until it goes into water. A summary of what was done must be submitted by email in a post-treatment Monitoring Report within 24 hours of intervention and should minimally include:

1. Date of intervention
2. Names of all responder(s) and roles in intervention
3. Intervention start and finish times
4. Type(s) of intervention performed (*i.e.*, lanced abscess, administered antibiotics) and photos/video if possible
5. Details of intervention (tools used, type of restraint)
6. Name and volume of any drug(s) administered
7. Location (body site) and route (presumably IM) of drug administration
8. Follow-up observations made during immediate post-intervention monitoring (30-60 min. post)
9. Description of any complications that occurred during or after intervention
10. When animal was next observed:
 - a. Date
 - b. Behavior
 - c. Appearance of injection site (photos if possible)
 - d. Appearance of wound/injury/abscess (photos if possible)

CLEAN PROTOCOL FOR HANDLING SEALS:

When handling Hawaiian monk seals, our goal is to limit the possibility of disease transfer either to humans or to other seals via any of the five basic routes; direct, aerosolization, ingestion, injection, and absorption. **Proper sanitation can prevent or minimize transmission.**

Below are general procedures to follow whenever there is physical contact between humans and live monk seals.

- 1. Protective Clothing:**
Seal restrainers (*i.e.*, those who may come into contact with the seal's body) should wear clean coveralls, disposable latex or nitrile gloves, and full coverage footwear. Cloth gloves over the disposable gloves are optional, and may provide better grip. Protective eye wear (*e.g.*, sunglasses) and kneepads are recommended, but optional.
Other response personnel (*i.e.*, those that will not contact the seal) should wear disposable latex or nitrile gloves (if handling samples), eye protection and full coverage footwear.
- 2. After Each Capture:** **CLEAN** all instruments/gear by washing thoroughly with soap and water. This includes nets, instruments, buckets, and other items that may have been contaminated. Once cleaned, **DISINFECT** everything with a 1:20 solution of bleach water* (can use salt water) for a minimum of ten minutes. Place all sharps in a labeled "SHARPS" container. Any contaminated non-sharps disposable items (*e.g.*, gloves (latex or nitrile), ziplock bags, and garbage bags (holding soiled protective clothing), may be bleached and disposed of in the trash or put in a biohazard bag inside of a five gallon bucket labeled "For biohazard use only".

Rinse all equipment after the 10 minute bleach soak. Thoroughly WASH HANDS with anti-bacterial soap.

- 3. Back At Camp:** WASH and then SOAK all coveralls, cotton gloves, and other reusable cloth gear (including footwear) for preferably one hour but at least 10 minutes in bleach solution (1:20) and then rinse and let dry.

Bleach solution may be re-used within a 24-hour period (unless it is contaminated with organic matter). Do not dispose of the bleach solution into the ocean. Once the solution no longer smells like bleach, the bleach has evaporated and the remaining liquid can be poured into the sand above the high water line.

SEAL HANDLING

- 1. When to handle and time of day.** Because older animals are larger, they have less surface area relative to body mass, and are more susceptible to heat stress. **Have water ready to cool the animal** and, limit your efforts to **early morning or late afternoon/evening**.
- 2. Disturbance.** The seal to be handled should be away from other seals.
- 3. Mental preparation.** Before handling/tagging any seal, even a pup, make sure you are completely prepared. Double check all equipment and supplies, talk the team through the event, assign roles, discuss what to do when, and ensure all are mentally prepared. Make sure you have a bucket of water handy, or someone is designated to get the water as soon as the procedure begins.
- 4. Environmental hazard assessment.** As mentioned above, there are several external factors, such as ambient temperature, that need to be considered prior to catching a seal. Take at least a minute to survey the seal's surroundings for any hazards that might threaten you or the seal during capture. In particular, look for anything that might injure the seal if it thrashes or resists restraint. This could include sticks, rocks, or different types of debris. If a weaned pup is captured in the wave wash or close to the water's edge, you can move it up the beach - but not great distances (a few meters) by dragging it by its rear flippers or putting it in a stretcher net and carrying it away. If you are using a net, be aware of the seal's proximity to the water and the potential for the seal getting to the water while entangled in the net. If the threat cannot be mitigated, then wait for the seal to move or try herding the seal to a more favorable spot. Be conservative when assessing threats, the seal can always be captured at another time and it isn't worth injuring yourself or the seal. **If you, or anyone on your team, have doubts, SPEAK UP.** A good rule of thumb: If there is anything that makes you question your safety (*i.e.*, you might cut yourself catching a seal on a rocky platform) it is probably a good idea to catch the seal somewhere else.
- 5. Seal capture and position.**

Recommended minimum number of team members for animal handling by size of seal:

Weaned pup: 2 people, may/may not use a stretcher net
Juveniles: 3 people, and a stretcher/hoop net
Subadults/adults: 4 people, and a hoop net

Before capture, record the time of day. The first person on the seal is the head restrainer, who is the one to direct the handling, and is the last person off the seal. Use low voices and do not walk or move in front of the head. Monk seals are not aggressive, and their first response is usually to flee. However, all seals may bite, including pups (though their teeth may not be fully erupted yet). **A restraint**

time limit of 10 minutes or less is recommended, however the appropriate time is dependent on how hard the animal is fighting, how hot it is, the condition of the restrainers, etc.

The restrainer will capture the seal, but may require help from the rest of the team. The restrainer should straddle the seal's body at the shoulders, and use his/her hands and knees to hold the animal in position. Put as little weight on the seal as possible - most of the time you won't need to put any weight on it. **Don't put your full weight on the seal. Do not stand or kneel on the seal's flippers.** Be particularly cautious that there are no rocks or other objects that could obstruct the seal's airway beneath the neck. Restrain the fore flippers by folding them along the animal's side, stabilizing them with your knees and thighs so it can't use its fore flippers as leverage to roll. The seal should be laying on its ventral surface. The seal may try to roll, and it will be the restrainer's job to reposition the seal on its ventral surface. It is helpful if you can maintain the seal with the head facing uphill. You may find it easier to control the head if you grab the skin folds of its neck. Other staff can help control movement of the hips and hind flippers as necessary. It is not absolutely essential that the seal be flat on its abdomen, though this position is safer for the handler. Remember to stop timing at the end of the event.

Handling/tagging of older animals is more difficult because the animals are larger and stronger, and therefore **more difficult to restrain**. They therefore represent a **more serious threat to the restrainer(s)**, and are capable of inflicting more serious injuries. In some instances the restrainer may not straddle the animal, but will lie along the sides of the seal's body holding the seal down (like a human squeeze cage).

6. Seal stress.

- a. **Restraint stress.** Restraint times for treatments should be kept as short as possible to minimize stress to the seal. The head restrainer is responsible for monitoring the seal's level of alertness and respirations throughout the restraint period. The seal's breathing pattern may be irregular, and it may only breathe through one nostril. However, if there is a sudden change in breathing pattern, either a rapid increase or sudden decrease, this raises concern. For example, if a pup holds its breath for more than 20 seconds, immediate release should be considered. Check the seal's eyes to see if they are responsive; i.e., is the seal looking around, does it respond to your hand or something that you move into its field of view? Tap it gently with your finger behind the eye. If it doesn't show some response or its response is slow and the seal does not appear to be attentive, then abandon the procedure and immediately release the animal and monitor it from a distance. **Please be conservative in your decision-making and err on the side of caution.** You can capture the seal again later if you need to (under the direction of the vet); however, if it dies due to capture stress, this will negatively impact not only the individual seal and species recovery, but our ability to

conduct similar treatments in the future.

- b. **Heat stress.** Because procedures are usually relatively quick and the pups are relatively small, the chances of heat stress are reduced. Nevertheless, heat stress is possible and potentially lethal, especially on a hot afternoon and for larger/older animals. Make sure you have water handy to cool the animal. The animal is best cooled by gently pouring water over its hind flippers. Don't sit on the animal. Try to minimize the amount of its surface that is covered with a warm human body. If in doubt, always err on the side of keeping a seal cooler, as cooling an overheated seal is difficult and often unsuccessful.

Hawaiian Monk Seal Antibiotic Treatment Protocol

PIFSC staff must document information in the table below for all field staff authorized and trained to administer antibiotics to Hawaiian monk seals

Only State or Federal employees (no volunteers) are authorized to administer antibiotic medications to wild monk seals (via pole syringe or otherwise), and only after consulting with a veterinarian.

Individuals that may be authorized to administer antibiotics via pole syringe or otherwise in the NWHI during the 2012-2013 winter field season are listed below.

Island/Atoll	Name	Agency	Title	Training/previous experience
Laysan	Andrea Kristof	USFWS	Biological Science Technician (field camp leader)	HMS abscess treatment/pole syringe training 9/27/2012.*No direct previous HMS experience, although has worked at Laysan multiple years.
FFS	Chad Bell	USFWS	Tern Island Refuge Manager	HMS abscess treatment/pole syringe training 10/2/2012.* No previous HMS experience. Has used pole syringe to inject antibiotics into elk several times.
Kure	Matt Saunter	DLNR Hawaii/RCUH	RCUH/CCRT/Natural Resources Management Technician (Kure DLNR field camp leader)	HMS pole syringe training 10/09/2012.* Has assisted with HMS tagging and disentangling.

Island/Atoll	Name	Agency	Title	Training/previous experience
Kure	Naomi Worcester	DLNR Hawaii/RCUH	RCUH Biological Technician (Kure DLNR field camp assistant)	HMS pole syringe training 10/09/2012.* Worked previously for HMSRP at Kure, has treated HMS abscesses in the field, including injecting antibiotics into restrained animal on 4 consecutive days.
Kure	Ilana Nimz	DLNR Hawaii/RCUH	RCUH Biological Technician (Kure DLNR field camp assistant)	HMS pole syringe training 10/09/2012.* Worked previously for HMSRP at Kure, has treated HMS abscesses in the field, including injecting antibiotics into restrained animal on 4 consecutive days.
Trained, but not currently scheduled to deploy	Cynthia Vanderlip	DLNR Hawaii/RCUH	RCUH Biological Field Station Supervisor	HMS pole syringe training 10/09/2012.* Has previous experience tagging, restraining, translocating, disentangling HMS

Individuals listed below also attended the training but are not authorized to inject antibiotics.

Tern:

Olivia Bailey Tern Volunteer
 Larry Chlebeck Tern Volunteer
 Abram Fleishman Tern Volunteer

Laysan:

Royce Daniels Laysan Volunteer
 Leslie Parker Laysan Volunteer
 Andy VanDeusen Laysan Volunteer

Kure:

Joshua Willman Kure Volunteer
 Dakshina Marlier Kure Volunteer
 Parker Shebs Kure Volunteer

***Description of training:**

All individuals were trained by Angie Kaufman, who has treated abscesses in the field, including injecting antibiotics. She was trained by Dr. Gregg Levine in using the SafeTFlex pole syringe and was trained and supervised by Dr. Bob Braun to administer injections to live animals using a regular syringe. All trainees were instructed to contact NMFS if an abscess is suspected, and to take no action without consulting with a designated NMFS veterinarian. Training included introduction to equipment and supplies, importance of human safety, sterile techniques when administering antibiotics, and how to administer antibiotics via regular and pole syringe. Training refresher courses will occur annually in conjunction with field staff training. Pole syringe training included instructions following the directions below:

SafeTFlex pole syringe - Attach a sterile needle of appropriate size (16-18g, 2-3.5" long, depending on size and estimated blubber thickness) to the pole syringe. Draw up appropriate amount of antibiotic into the syringe. The drugs are administered through the pole syringe by the force used to push the syringe into the dorsal posterior end of the seal. If a repeat dose is authorized, administer it on the opposite side of the body from the first dose. If possible, wait until the seal is sleeping and sneak up behind the seal to give the injection in the right gluteal region. Follow through with a forward motion until you are certain the full dose of antibiotics is given. Let the motion of the seal as it moves away from you withdraw the needle. If available and ONLY if it will not interfere with the response effort, you may use a pole-mounted camera (or camera on the responder's head) to video the effort for assessment and debriefing purposes. Alternatively, photograph the animal as soon as possible after the response for assessment of the injection site location.

Drug dosage to be used with permission of veterinarian:

Ceftiofur (Excede, 200mg/ml) dose is 6.6mg/kg

See Abscess Treatment Protocol for directions on how to administer Ceftiofur.

PIFSC staff must document the following information if, under the direction of a veterinarian, it is determined that antibiotics should be administered to a HMS.

- Date of initial contact
- Island/Atoll
- Names of individuals responding
- Background information on situation
- Training-include summary of information covered in training, who did the training, who attended (see table above)
- Attending vet
- Action taken

- Drug dosage and precise description of injection site location
- Monitoring Report-report on what was done, how animal responded, follow up observations

File No. 16632 Appendix E: Hawaiian Monk Seal Epidemiology Sampling and Sample Processing Protocols

Hawaiian Monk Seal Epidemiology Sampling Protocol

All persons coming into direct contact with the seal or collecting specimens must be in good health and **wear gloves**. Eye protection (sunglasses or other) is recommended (see “Tagging and Handling” section of HMS manual). Before handling a seal, a vet assistant should be identified and given instructions.

TOOLBOX SUPPLY LIST:

Bleeding supplies:

6ml and 12 ml syringes
18g x 1.5” needles
18g x 3.5” spinal needles
Pre-assembled vacutainer hub/adapters in clean ziplocs
Extra vacutainer adapters
7.5 ml LTTs
3ml LTTs
10ml GNTTs
10ml RTTs
PAX gene RNA tubes

Biopsy supplies:

Sterile surgical gloves (sizes 6.5, 8.5)
Gauze
6mm Acuderm biopsy punches
5ml cryovials
2.5 ml precleaned teflon vials (NIST)
Precleaned instruments (NIST) wrapped in foil (scissors, forceps)

Fecal supplies:

C&S media & swabs

Viral swab supplies:

Dacron fiber tipped swabs w/ plastic applicator
1.8ml cryovials pre-labeled with 2 each: eye, nasal, oral, rectal, genital

Additional supplies:

Crash kit with fluids and emergency drugs (See Appendix A)
Pole syringe
Sedative(s) – at veterinary discretion (midazolam, concentrated midazolam, diazepam)
Cooler and blue ice
Towel
Ceftiofur (Excede)

25 or 30 mL syringes
16g x 3 ½” needles
Powder free gloves
Data sheets: Epidemiology Sampling Form, Tagging Data Sheet
Clipboard, pens for labeling, pencils for data recording
Betadine-soaked gauze
Alcohol-soaked gauze
Teflon squirt bottle of high purity alcohol (isopropanol)**
Tape measure
Sharps container
Trash bag/container
Rectal thermometer w/ extra batteries
Sterile lubricant (KY gel or other)

****Always replace teflon bag over squirt bottle! Please be mindful that this is an expensive bottle to replace and should be used specifically for this purpose.**

ORDER OF FIELD SAMPLING PROCEDURES:

1. Sedation and restraint
2. Blood collection order:
 1. Fill big (7.5mL) LTT ***ALWAYS FIRST**
 2. Fill one of each RTT and GNTT
 3. Fill PAX gene tube
 4. Fill remaining RTTs and GNTTs in any order
 5. Fill small (3ml) LTT
3. Swab collection (eye, nose, mouth, genital, rectal, fecal culture)
4. Blubber biopsy
5. Tag(s)

SAMPLE COLLECTION:

I. PATIENT MONITORING

A veterinarian must be present for all procedures requiring chemical immobilization. During restraint, vital signs, particularly respirations, should be monitored continuously. In the event of an emergency, the attending veterinarian will abort all sampling efforts and direct emergency procedures. One individual should be designated as the veterinary assistant and tasked with vital sign monitoring/communication with the veterinarian and assistance in the event of an emergency (drawing up emergency drugs, etc., see Appendix A).

II. SEDATION

Positioning: ventral recumbency with foreflippers tucked to the sides.

1. Diazepam (Valium) (5mg/ml): 0.1-0.25 mg/kg IV

- *Route:*
 - Diazepam is most effective when administered IV.
 - Use the extradural or hind flipper veins (see Fig. 1).

- Draw up appropriate amount of drug in a sterile syringe (a 6mL syringe is usually best).
- *Preparation:*
 - Clean the area with betadine solution and alcohol-soaked gauze.
 - Last, do a final spray rinse with the high purity alcohol in the teflon vial. This step is important for ensuring that NIST blood samples are not contaminated and are collected consistently.
- *Venipuncture:*
 - Palpate the vertebral column and pelvis and move your fingers cranially 2 or more vertebral spaces, feeling for a “divot” lateral to the spinous processes of the vertebrae.
 - Needle choice:
 - Pups and thin/average weaners: use an 18 or 20g x 1 ½ to 2” needle.
 - Adults and fat weaners: use a 3.5” spinal needle.
 - Before insertion, remove the stylet, holding needle from hub only.
 - Inform restrainers that you are ready to insert the needle.
 - The angle of the needle may vary from a 45 - 90 degree angle to the dorsal surface of the animal.
 - As the needle is inserted, feel it moving through skin, blubber, and muscle until you feel it pop through the membrane of the extradural sinus. You should now see blood rising to the hub of the needle. Attach the syringe containing the diazepam and inject.
 - Draw back using the same syringe to collect 2-3ml of “waste blood” and dispose of syringe/blood in the sharps container. It is collected to avoid contaminating blood samples with residual diazepam from the needle.
 - Remove syringe and attach the vacutainer hub/adaptor setup to the needle.
 - Fill tubes in order specified above.

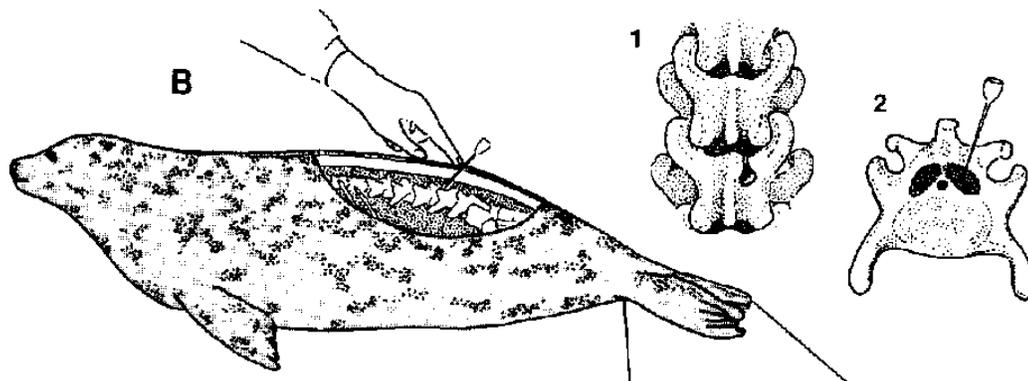


Figure 1.

2. Midazolam (5 mg/ml or 50mg/ml): 0.1 – 0.15mg/kg IM

- Some situations may require capture and manual restraint before drug administration. Other times, it may be sufficient to sneak up to an animal, administer the injection be prepared with boards/nets in case the animal approaches the water. The decision to restrain or corral an animal is to be made between with the handling team and veterinarian on a case-by-case basis.
- *Route:*
 - Midazolam should be administered IM. Draw up the appropriate amount of drug in a small syringe for accuracy.
 - Pole syringes are ideal for IM drug administration, as you can sneak up on a sleeping animal and inject in the hindquarters rapidly, causing minimal stress.
 - Instructions for loading the Dan-Inject Jab Stick pole syringe are included in the syringe carrying case.
 - Special needle sizes (16g x 3 ½” needles) are available for use with large adults.
 - For hand injections, transfer appropriate dose to a large (20mL or larger) syringe to speed drug administration. Use an 16 to 18g x 2“ needle.
- *Administration without immediate restraint:*
 - As the vet approaches the seal to administer sedation, 2-3 people with boards should be standing by (quietly, low to the ground and as out of sight as possible). Ideally, the animal’s reaction to the injection will be brief and if everyone else remains beyond the flight distance of the seal, the best scenario is that it will not move far and go back to sleep.
 - If the seal moves toward the water (or other danger such as boulders, ledges), boarders should approach the head of the animal to prevent the animal from it from reaching the danger or water.
 - A person with a net should be ready in case the boarders cannot contain the animal adequately.
- It will take 10-15 min. for the sedation to take effect.
- Record respiration rate and activity (movement, head position, etc.) at least every 5 min. on the Epi Sampling Form. Use these trends to gauge the alertness of the seal. Do not approach the animal too soon after administering the sedative.
- Once you approach the animal, do so quietly. Consider wetting down a towel and place it gently over the seal’s eyes (only), as this will help keep it calm. Proceed to blood sampling.

3. Reversal of sedative: Flumazenil (0.1mg/ml): 0.1 - 0.2mg/kg IM or IV

- Midazolam and diazepam can be reversed if an accidental overdose is given, if the animal is not responding well to the sedation and emergency procedures are instituted, or if the animal is too slow to wake up following a procedure. IV administration will work rapidly, but IM administration is also fast (minutes) and can be used if IV access is not immediately available.

III. BLOOD SAMPLING

- *Assistant duties:*
 - Before capture, set the vacuums in the RTT and LTT vacutainer/syringe tubes by pulling back on the plunger until it locks into place and snapping it off. This step should **not** be done at the lab ahead of time, as syringes can lose their vacuum.
 - The assistant should ensure that the veterinarian receives the blood tubes in the correct order (see below).
- Attach vacutainer tubes (they will automatically fill if you are in the vessel).
- Fill tubes at least halfway to allow for proper ratio of blood to anti-coagulant.
- Gently rock the tubes 10-15 times to thoroughly mix additive and blood.
- Fill tubes as follows:
 1. Big (7.5mL) LTT
 2. One of each RTT and GNTT
 3. PAX gene tube
 4. Remaining RTTs and GNTTs in any order
 5. Small (3ml) LTT
- Immediately place all tubes **upright** (in particular, the RTTs) in the styrofoam tube holder in the cooler. Tubes should not come into direct contact with the ice, which will cause blood cell lysis.
- The PAX gene tubes must be cooled gradually. They should be stored upright at room temperature for 2 hours before freezing (in the cooler with blue ice is fine, provided that they do not come in direct contact with ice). When freezing, the PAX gene tube should be frozen for 24 hours at -20C before being transferred to the -80C freezer. Long-term storage in a -20C freezer is acceptable as well.
- If the large LTT is not filled first, please note this on the datasheet.

III. SWAB COLLECTION

Use sterile dacron polyester fiber tipped swabs with plastic applicators. Swabs should be collected from the following locations: rectum, genital orifice, nares, medial canthus (corner) of eye, lateral commissure (corner) of the mouth. Collect 2 swabs from each location, place in pre-labeled cryovials, break off tips of swabs in sterile manner, and preserve in liquid nitrogen.

Collect 2 fecal swabs and place both in one vial of C&S media.

IV. BLUBBER BIOPSY

Preparation of biopsy site

Wear sterile surgical gloves. The blubber biopsies should be collected from the lateral aspect of the seal's pelvic girdle, 5-15cm cranial to the wing of the ileum. Before inserting the punch, clean the area with betadine and 70% isopropyl alcohol (take precautions in windy situations to avoid getting in eyes of personnel). **After scrubbing, do a final rinse of the area with the high purity isopropanol in the teflon squirt bottle** (This is important for NIST sampling).

Blubber biopsy collection and disposition

Collect two full-thickness biopsies (2-5 cm). Use the pre-cleaned, foil wrapped (provided by NIST) thumb forceps or scissors as necessary. **Do not allow gloves to contact tissue.** After samples have been collected, clean the biopsy sites with betadine solution. The wound can be left to heal through second intention.

The first blubber sample should be stored in a 7mL teflon vial for toxicology analysis. The second sample should have the skin removed (as aseptically as possible). The skin should be placed in a 2mL cryovial containing DMSO. The blubber should be placed in a cryovial for fatty acid radioactive isotope analysis. Freeze both samples in liquid nitrogen as soon as possible.

SAMPLE COLLECTION GUIDE

See Sample Processing Protocol for details on what to do back at the lab.

Item (#/seal)	Tube Vol.	Anti-coagulant	Blood Fraction	Short-term	Processing instructions	Long-term	Purpose (Investigator)
LTT (1)	7.5 mL	EDTA	Whole	Blue ice	Divide into 1mL aliquots and place in cryovials	LN	Archive (NIST/Trace Elements)
LTT (1)	3 mL	EDTA	Whole	Blue ice	IDEXX	Do not store	Blood chemistry (HMSRP)
PAX gene tube (1)	2.5mL	RNA preservative	Whole	Store upright at room temp for 2 hours	Divide into 1 or 2mL aliquots in cryovials after 2 hours at room temp. Then transfer to -20C freezer for 24 hours. Then transfer to LN/UF if desired.	-20/ LN/ UF	Biotoxins (NOS)
GNTT (3)	10 mL	NaHep	Plasma	Blue ice	a) Spin down, transfer 2.5 mL from tube into 7 mL Teflon jar b) Divide remainder into 1 mL aliquots and place in cryovials	LN	a) Contaminants (NIST) b) Archive (min. 4 aliquots each to NIST & HMSRP)
GNTT (1)	4 mL	NaHep	Plasma	Blue ice	Spin down and aliquot into cryovial(s)	LN/ UF	a) Biotoxins (NOS)

Item (#/seal)	Tube Vol.	Anti-coagulant	Blood Fraction	Short-term	Processing instructions	Long-term	Purpose (Investigator)
RTT (4)	10 mL	None	Serum	Upright Blue ice	Divide into 1mL aliquots and place in cryovials	LN/ UF	a) Tier 1 (HMSRP) b) HMSRP/ NIST Tissue Bk (minimum 4 aliquots each to NIST & HMSRP)
Viral swabs				Blue ice		LN/ UF	Tier 1 (HMSRP)
Rectal swabs				Blue ice		Fridge	Culture (<i>Vibrio</i>) (UC Davis)
Blubber biopsy #1				7 mL vial (Teflon) Blue ice		LN/ UF	Contaminants (NWFSC)
Blubber biopsy #2				2 mL cryovial	a) Remove skin from biopsy at the lab and place in DMSO cryovial b) Freeze biopsy	LN/ UF	a) Skin: archive/stable isotopes (HMSRP) b) Fatty acids (S. Iverson/Dalhousie; CITES permit required)

Hawaiian Monk Seal Sample Processing Protocol

LABELING

Use nalgene cryoware markers for labeling vials. Label all samples as follows:

Seal ID Collection date (YYYYMMDD) Specimen # and subnumber (ex: 1000A) Specimen Type – Subtype (ex: PS-GT) HMS Island

Specimen Types: PS - plasma; SE - serum; WB - whole blood; BB - blubber biopsy

Specimen Subtypes: GT - green top; LT - lavender top; RT - red top

Please refer to the cheat sheet in the lab for additional sample codes.

IDEXX Samples only need to be labeled with Seal ID, Date, Specimen # and Account #1689 (for routine clinical samples; see Angie for IDEXX research account number if applicable).

BLOOD TUBE PROCESSING

Note: Whenever you start a new lot number of blood tubes, you must make a field blank for NIST. See Epidemiology Sampling Protocol for instructions.

1. Every effort should be made to process blood **within 4 hours of collection**.
2. If sending samples to IDEXX, call 1-800-444-4210 and tell them you are calling from acct #1689 and have samples to be picked up. Confirm pick-up time. See IDEXX Shipment Instructions for more details.
3. Put on a clean pair of powder free gloves, disinfect your work surface and/or put down one of the tex wipes provided by NIST. Refer to biosafety documents.
4. Brush sand off of tubes and make sure tubes are properly balanced in centrifuge (use water-filled tubes to balance as necessary). Spin the RTTs and GNTTs for 10 minutes at 2000 rpm.
5. While RTTs and GNTTs are spinning, assign specimen numbers and ensure that samples and containers are labeled:
 - Cryovials w/ swabs
 - Blubber biopsies
 - Cryovials for serum, buffy coat and plasma aliquots
 - C&S media for fecal culture
6. Place each animal's samples in a separate cryobox. Then put the plastic box in a plastic bag along with the two Teflon vials. Label bag with seal ID and date, and then place in UF. Record location of cryobox on specimen data sheet.

Lavender-top EDTA tubes (whole blood):

7.5mL tube

- Wearing clean gloves, unscrew the blood tube top
- Pour 1mL aliquots of whole blood into 2mL cryovials. To prevent contamination, do not remove aliquots from these vials.
- Label each aliquot as directed above
- Place in cryobox and transfer to UF

3mL tube

- Label tube as directed above.
- Refrigerate.
- Send to IDEXX (see IDEXX Shipment Instructions)

Red-top tubes (serum):

- Using a clean, plastic pipette, place 1 mL aliquots of serum into 2 mL cryovials.
- Be sure to record states of hemolysis and/or lipemia in the notes column of the specimen log and lab data sheet.

- Assign specimen numbers and label serum vials as shown above.
- In most cases, 1.0 ml of **refrigerated** serum will be sent with the 3mL LTT to IDEXX for chemistry. **Use serum sub # E.** See IDEXX Shipment Instructions.
- Place remaining serum aliquots in cryobox and store in UF.
- Dispose of remainder of RTT after all serum is collected.

PAX gene tube (whole blood):

- After blood is collected, rock tube gently to mix additive with blood.
- Store at room temperature for at least 2 hours (up to 72 hours).
- Transfer to 1 or 2mL cryovial aliquots and place in -20C freezer for at least 24 hours.
- Long-term storage in -20C or -80C freezer is appropriate. If storing in -80C freezer, samples should be frozen at -20C for 24 hours before transfer to -80C freezer.

Green-top tubes (buffy coat, plasma):

- Transfer plasma from the 4 mL GNTT into a cryovial for biotoxin analysis. Assign specimen number and label cryovial. Freeze in UF or LN.
- Using a NIST-provided pipette, transfer 2.5 mL of plasma into a 7 mL Teflon jar (Contaminants). Label the side of the jar and the paper Nomex tab and place tab in recessed lid of the Teflon jar.
- Use a clean glass Pasteur pipette to aliquot 1mL of plasma into 2mL cryovials.
- Then, re-spin the tube in the centrifuge for 10 min.
- Use a clean plastic pipette to transfer the buffy coat to a separate cryovial.
- Place all samples in a cryobox and store in UF.

OTHER SAMPLES:

The C&S medium sample should go in the refrigerator to be shipped to UC Davis.

Emergency contact #s:

Liz work c 721-5344, personal c 285-4578

Angie work c 343-6249, personal c 512-773-7341

Dr. Bob Braun 783-6565

Dr. Gregg Levine 358-5311

Dr. Michelle Barbieri c: 443-834-8612, w: 808-983-3706

SAMPLE SUMMARY

Bold = Tier 1 Sampling

LAB	Screening
IDEXX	CBC/chemistry
Jerry Saliki, Athens Diagnostic Lab, UGA	Morbillivirus serology and PCR
Terry Spraker, CSU	Histopathology
J. Dubey, USDA	Toxoplasma serology
WADDL	Canine Adenovirus, Feline Calicivirus and in MHI, Parvovirus
Athens Diagnostic Laboratory, UGA	Phocid herpesvirus 1 serology
NVSL	Chlamydomphila psittaci
Renee Galloway, CDC	Leptospirosis
UC Davis	Fecal culture
Mike Grigg, Nat'l. Institutes of Allergy/Inf. Dz	Toxoplasma PCR/genotyping
Marine Biotoxins Program, NOS	Biotoxins
Jenny Schultz, UH Manoa	Genetics
Mote Marine Lab	Fertility potential
Mystic Aquarium	Brucella (cELISA serology)
NVSL	Brucella (culture and isolation if indicated)
Jeff Stott, Tracy Goldstein (UC Davis)	Herpesvirus
NIST, archive	Toxicology

File No. 16632 Appendix F: Health Screening and Quarantine Protocols for Translocations

HEALTH SCREENING AND QUARANTINE PROTOCOLS FOR HAWAIIAN MONK SEAL TRANSLOCATION BETWEEN SUBPOPULATIONS

These protocols support NMFS' translocation actions. These protocols are intended for any seal translocations between subpopulations (e.g., two-stage translocations or experimental juvenile translocations), as opposed to rapid and short distance translocations (within atolls or within the main Hawaiian Islands, MHI). Separate protocols are included for translocating different age classes of seals and are applicable to any locations in the Hawaiian Archipelago.

These protocols are subject to refinement and change based on experience that will accrue during the next decade, veterinary consultation, emergence of new testing procedures, disease risks, etc. Protocols will be reviewed annually and updated as required to refine protocols and improve implementation.

Weaned Pup Translocations

Steps involved in weaned pup translocations include:

- 1) Selection and capture of seals, health screening, and attachment of tracking instruments.
- 2) Recapture and transport to vessel/aircraft.
- 3) Transport to destination site.
- 4) Release of seals at new location.
- 5) Post-release monitoring.

Transport Vessels

A variety of transportation modes will be used including large vessels (NOAA ships, other chartered vessels), airplanes, helicopters, automobiles, and other as appropriate depending on location and available resources.

Specific Protocols

- 1) *Selection and capture of seals, health screening and attachment of tracking instruments.*

Any weaned pup at the designated source site will be considered a candidate for selection, as long as it exhibits no apparent signs of disease, injury or any other factors that may compromise survival. Relatively recently (i.e., less than a month previous) weaned pups may be favored for selection as they are more likely to remain at the release location longer than those that have weaned earlier (Baker et al. in review). Seals will undergo health screening and a subset will be instrumented with a tracking device approximately 1-4 days prior to transport. Seals will be captured using standard practices (by hand or using a hoop net). Blood may be collected without sedation or seals will be sedated.

Seals will be evaluated using the current standard health screen. This may be modified as deemed necessary due to specific disease concerns in source and recipient subpopulations, up to date testing procedures and veterinary consultation. Current practice includes:

Blood Analysis

1) Field analysis:

- a. WBC count – Unopette system
- b. RBC count – Unopette system
- c. WBC differentials, platelets – Microscope and archive extra unstained smear
- d. Hematocrit/ PCV – Microhematocrit centrifuge
- e. Hemoglobin
- f. Serum chemistry (Na, K, Cl, BUN, Creat, Ca) – I-Stat kit
- g. Glucose – Glucometer and test strips
- h. BUN - Azostix

2) Lab analysis (frozen 0.5-1.0 mL aliquots of serum, stored in liquid nitrogen dewar in the field)

- a. Serum chemistry – send to IDEXX
- b. Tier 1 testing, which currently includes: morbillivirus, seal herpes 1, Toxoplasma, and fecal culture.

3) Banked blood samples stored in liquid nitrogen dewar in the field

- a. Remaining serum (or at least 4 aliquots)
- b. Whole blood (Na heparin and EDTA)
- c. EDTA plasma, buffy coat, and RBC
- d. Na heparin plasma, buffy coat, and RBC
- e. PAX gene blood RNA tube (for biotoxins)

Swab processing

1) In the field place all swabs in the liquid nitrogen dewar after collection

2) Lab analysis

- a. 1 nasal and 1 rectal swab in Avian Influenza transport media (frozen) – send to National Wildlife Health Center in Madison
- b. 2 dry swabs from the eyes, nares, mouth, genital orifice, rectum and any external wounds
- c. 1 swab of any abnormal tissue in viral transport media (if deemed appropriate)

Blubber Biopsies

Put in liquid nitrogen dewar in the field

- 1) 1 for toxicology (Teflon container)
- 2) 1 for fatty acid analysis (cyrovial)

Other Sampling

- 1) Any other sampling deemed necessary by the PI or attending veterinarian.

External Exam

- 1) Physical Exam
 - a) Assessment for external wounds
 - b) Auscultation of lungs, heart
 - c) Examine eyes, nose, ears etc. (damage, disease, moisture)
- 2) Morphometrics
 - a. Girth
 - b. Length
 - c. Weight

Samples not analyzed in the field will be stored, shipped, and analyzed as described in the current monk seal permit.

If, based on veterinarian's physical exam and immediately available test results, seals do not show any signs of injury or illness, some may be instrumented with appropriate telemetry equipment to monitor them after release. This device will assist post-release monitoring until the opportunity to visually survey the seals arises.

If seals do show physical signs of injury or illness, the attending veterinarian will determine whether to sedate for full biomedical sampling or to treat the injury or illness. These animals will be covered under the health assessment portion of the PIFSC research and enhancement permit, or under the MMHSRP permit depending on the treatments required.

After this handling, seals will either be released and allowed to freely range until capture for transport, or will be held in a shore pen (approximately 1-4 days). Allowing seals to freely move will minimize any stress seals may experience being held in a captive shore pen. Holding in shore pens allows for better assessment of animals health and reduces effort of relocating seals within the atoll. The decision to use pens or allow seals to free-range prior to transport will depend on conditions at the field site, results of physical examination and transport logistics. If seals are allowed to range freely, prior to the second capture the seals will be visually assessed for any outward signs of injury or illness. If the attending veterinarian determines the animal to be unhealthy, either after physical examination and/or evaluation of blood sample, then the animal will not be translocated.

2) Recapture and transport to vessel/aircraft.

Weaned pups will be captured using standard techniques for the transport of weaners. If transport involves a small boat shuttle to a larger ship, animals will be restrained in a stretcher net by two trained seal biologists and placed on the deck inside the small boat. Seals will then be transported directly to the vessel. Water will be available onboard to cool the seal when needed. The number of seals that may be transported at one time in the small boat will be dependent the specific boat's capacity.

There should be adequate area that no seals are piled on top of each other and that there is a reasonable amount of space for researchers to operate to cool and move seals as necessary.

Seals will be taken onto the vessel by lifting the entire small boat by crane up to the mid-ship low railing access on the port side of the vessel (or the safest method depending on the vessel being used). One biologist will remain with the seal during lifting. Seals will be hand lifted from the small boat onto the vessel and brought to their cages.

The distances between cages will be wide enough to allow biologists to move between, prevent spread of urine and feces between cages, and allow the free flow of air. The cages will be strapped to the deck to prevent sliding if rough seas develop. Seals will be placed on a blue tarp, removed from the stretcher net and lifted manually into the cages. Seals will be held separately. A saltwater hose is located near the cage and ice is available for cooling off seals in the heat of the day. Cage openings will be accessible to allow access to animals if medical care or treatment is needed in transit.

If transport is via automobile to aircraft, similar but more logistically simple procedures will apply. Seals will be captured in the same way. Unless it is not feasible, the seals will be transported in cages (again while being observed and with water for cooling available) in automobiles and likewise aboard aircraft.

3) Transportation to destination site

The transportation of seals between subpopulations could be done via boat, plane, car, or other reasonable mode of transportation. Multiple modes of transport can be used at any time. During all transports, the animals will be escorted by a veterinarian and sufficient staff to be able to respond to an emergency.

Transport via ship

During transport the deck(s) holding the seals will be off limits to anyone except seal biologist monitoring the animals, the veterinarian and ships safety officers. No physical contact with seals will be made unless a problem arises in which a seal needs to be restrained for examination or treatment (see contingency plan below). If physical contact is made, protocols for handling seals in the wild will be followed as described in the permit application and as written in the Hawaiian monk seal Field Research Manual for safe handling of seals and minimizing risk of disease transmission (e.g., clean coveralls that have been soaked in bleach solution, wash hands, etc). Observers will look for a variety of threats, indications of stress or disease, and ways to mitigate both while observing the animal:

- a) Entrapment/entanglement in cage
- b) Abnormal discharge from body orifices
- c) Abnormal respiration
- d) Abnormal behavior
- e) Modifying ambient temperatures to prevent overheating
- f) Enforce security-preventing disturbance by people on ship

g) Monitor for ship equipment/supplies posing risk to seals.

Seals will be monitored 24 hrs a day while on the ship. Observers will watch for changes in external behavioral/health parameters. Initially upon be loaded onto the boat the seals will be closely observed for signs of acute stress (e.g. continued high respiration and heart rate, agitated behavior, shaking). Descriptive and medical observations will be collected for each individual seal. The following types of data will be recorded:

- a) Observation form to be annotated at the end of each shift with significant findings; summary form to be completed by veterinarian daily.
- b) Summary form to be completed at the end of each 2-hour shift
- c) Eye exam form - only if eye issue is observed

Veterinary exam sheet will also be filled out by the attending vet prior to release.

4) Release of seals.

The protocols for releasing seals will be dependent on conditions at the selected release site(s).

General Considerations:

- Most releases will be on shore at a beach selected based on suite of criteria including, but not limited to:
 - site where pups have weaned and survived in past
 - ideally where conspecifics of similar age are present or frequent
 - if in MHI, then isolated from human contact
- Immediately after release seals will be monitored on shore for as long as logistically practicable.

If the site is a remote island or beach and landing by small boat is treacherous then this strategy will be considered (this will only be done in rare circumstances):

The vessel will approach the release site and attempt to get as close as possible to minimize distance traveled by small boats. Seals will be removed from their cages and placed on a blue tarp. They will be captured using a stretcher net and brought to the small boat, which will be held by the crane at the portside mid-ship low railing access (or other technique deemed safest and depending on vessel). Seals will be transported on the floor of the small boat and the boat will be lowered into the water for a near-shore release of seals.

The small boat will attempt to get within at least 100 m of shore but closer if conditions allow. This will mean the boat will be in shallow water with emergent land clearly visible for seals to navigate by. Two biologists will lift the seal over the rail of the safe boat, lowered to the surface of the water and one side of the stretcher net dropped allowing the seal to swim away. Safety lines will be tied to the boat side bar of the stretcher net and connected to the SAFE boat. This will keep the stretcher net from sinking and will cause the net to open releasing the seals if it should be dropped. An

additional crewmember will be prepared with snorkel gear to help in the water if something needs to be done in the water.

If the site can be accessed by truck or other vehicle the following should be considered:

- Time of transport should be minimized so animals should be moved be transported during peak traffic times
- Animals will be escorted in the back of the truck by monk seal specialists to monitor the animals' health and welfare during transport
- Water will be available to cool the seal during transport
- A veterinarian and emergency gear will be available should an animal need assistance
- A back up/escort vehicle will be accompany the transport in case a vehicle should breakdown, so the animal(s) can continue to be moved

5) *Post Release Monitoring*

a. Remote Monitoring

Movement and diving behavior of seals instrumented with tracking devices data will be compared to data concurrently collected from native seals or to pre-existing data on seals of similar age to determine whether translocated seal behavior is within the normal observed range.

b. Resighting

Attempts to resight translocated seals will be made during regular population monitoring effort or intensified observation at the release subpopulation. The level of observation effort will vary largely depending upon the accessibility, logistics and cost of mounting surveys. Subsequently, haulout behavior and survival of translocated versus native seals of the same age will be compared.

Translocation of Older Seals

The following protocols pertain to the translocation of juvenile or sub-adult Hawaiian monk seals (e.g., involved in the second stage of two-stage translocation). Similar protocols will be applied to translocation of aggressive adult male monk seals. Any seal older than 1 yr, which has been identified for translocation for any of the purposes proposed under the PEIS, may be subject to these protocols. Once identified for translocation, subjects will be considered further if they exhibit no apparent signs of disease, injury or any other factors that may compromise survival¹.

Steps involved in translocation of older seals may include some, but not necessarily all, of the following:

- 1) Selection and capture of seals for health screening and attachment of tracking instruments.

¹ Aggressive adult male selected for translocation to mitigate harm to other seals may nevertheless be selected even if compromised in some way.

- 2) Quarantine
- 3) Transport
- 4) Release of seals at new location.
- 5) Post-release monitoring

Transport Vessels (Same as for weaned pups)

Specific Protocols for Older Seals

1) Selection and capture of seals for instrumentation and health and disease screening. Procedures will be as described above for weaned pups with the following exceptions. Older seals will typically be captured with a stretcher or hoop net and transported in cages appropriate to their body size. Because older seals are far more mobile than weaned pups, they will usually be held in shore pens after initial capture until transport to the destination. As with weaned pups, seals which do not pass their health screen will not be translocated. If appropriate, they may be brought in for treatment under the MMHSRP or released on site if deemed appropriate by the attending veterinarian. Further, aggressive adult males deemed inappropriate for translocation may be brought into permanent captivity or euthanized according to the currently existing research and enhancement permit.

2) Quarantine Period

When transporting seals from the MHI to the NWHI, a period of quarantine may be necessary to reduce the likelihood of transferring a disease between the two regions. Quarantine holding will be done at a facility, on board a ship or in shore pens depending on the situation and facilities availability. The quarantine period should be long enough for the analysis of biomedical samples or longer than the prepatent period for the demonstration of clinical signs for the diseases of greatest concern. Two weeks is the generally accepted period and this period could include the transport period. Specific quarantine protocols are described in greater detail in a subsequent section.

3) Transportation to release site

Transportation of seals will follow the protocols established for weaned pups.

4) Release of seals at new location.

Release of seals will follow the protocols established for weaned pups.

5) Post Release Monitoring

Monitoring will be conducted as described for weaned pups.

Injury/Illness during transport

If during transport any seal becomes sick or injured, it will be cared for in transit by veterinary and husbandry staff equipped with emergency drugs, antibiotics, intubation equipment, fluids for hydration, and IQF herring if tube feeding is necessary. The compromised seal(s) will be monitored 24 hours/day until it can be delivered to a captive care facility. Captive care will be conducted using established protocols refined and developed with recent captive care activities for Hawaiian monk seals and other pinnipeds under the authority of the MMHSRP permit.

Eventual release of the seal will be determined according to standards of the MMHSRP.

Detailed Hawaiian Monk Seal Quarantine Protocol

The following are quarantine protocols that will be followed during the captive holding of Hawaiian monk seals, for example during translocation quarantine periods. Quarantine will typically occur in a captive facility, but these protocols can be adapted for use in a shore pen situation if needed. In such cases, reference to “pools” or “tanks” would apply to separate shore pens.

To date, no infectious disease that can be spread horizontally between seals has been found to cause clinical disease in Hawaiian monk seals. The following protocol takes this into consideration and is designed to reduce the risk of transmission of disease from outside sources to seals under human care. These sources include domestic animals and terrestrial wildlife (both directly and indirectly via fomites). Because humans act as fomites and because habituation of temporarily held monk seals is of paramount concern, every effort should be made to minimize human contact with releasable seals.

I. QUARANTINE

A. QUARANTINE DEFINITION AND OBJECTIVES

1. Quarantine refers to “any isolation or restriction on travel or passage imposed to keep contagious diseases, insect pests, etc. from spreading.”
2. Hawaiian monk seals held in captive care must be maintained under strict quarantine at all times. Quarantine measures between individual seals are at veterinary discretion based on health assessment findings.
3. All personnel involved in the feeding, handling, and care of these seals must be properly trained in quarantine procedures by an experienced staff. Quarantine procedures should always be clearly posted.

B. APPROVED DISINFECTING AGENTS

1. Dilute (10%) bleach, accelerated hydrogen peroxide or Nolvasan solution may be used. Practices differ slightly for each type of disinfecting agent and adherence to these practices is crucial to adequate quarantine.
2. The preferred agent is accelerated hydrogen peroxide (brand name: Accel) because it is less toxic than bleach and has a shorter contact time than bleach and Nolvasan.
3. CONTACT TIME is the most important aspect of disinfection. Each agent should be allowed to contact the surface that is being disinfected for the following minimum amounts of time:
 - a. Bleach: 10 minutes
 - b. Nolvasan: 10 minutes
 - c. Accel: 5 minutes
4. When using bleach, either in footbaths or otherwise, it is imperative that organic matter (feces, dirt, etc.) be removed from the surface FIRST. Bleach will not adequately disinfect in the presence of such debris.

C. NMFS QUARANTINE POLICY

Quarantine from Outside Sources

1. All equipment used in the quarantine facility, including feeding, handling, clothing and medical supplies **MUST** be:
 - a. Used exclusively for monk seals
 - b. Properly sanitized after each use
2. **NO VISITORS** are allowed in monk seal quarantine area unless previous approval is granted by the permit holder (Charles Littnan) and the on-site supervisor. This approval is granted on a case-by-case basis.
3. Any person working with wild or domestic animals or visiting another animal care facility on the same day must shower and change clothes before and/or after entering the seal enclosures.
4. Gloves should be worn anytime a seal (or biological samples) will be handled. Thoroughly wash hands with soap after handling seals or biological samples.
5. **FOOTWEAR:**
 - a. No street shoes are to be worn inside enclosures.
 - b. Closed-toe footwear designated for “monk seal quarantine” should be kept at the lower entrance to each enclosure. This footwear should be used in the enclosures at all times and nowhere else. Breathable footwear (such as crocs) is permitted unless the wearer will be in standing water contaminated with biological matter (*i.e.*, feces). Rubber boots should be worn to completely protect the feet from biological matter in these instances, such as during tank cleaning.
 - c. Footwear described above should be dipped in a footbath and scrubbed upon entry into and exit from the pool area. A footbath and long handled scrub brush should be kept at the bottom of the steps, inside the gate of each enclosure.
6. **PROTECTIVE CLOTHING:**
 - a. Any person that will potentially come in direct contact with seals must wear clothing that is designated for monk seal quarantine use only. This clothing can include coveralls, tee shirts and shorts/pants.
 - b. All quarantine clothing should be kept clean and remain at Ford Island in a designated area away from potential sources of contamination. It should never be worn when handling other species or animals outside of Ford Island.
 - c. Clothing should also be changed before and after handling any sick individual seals.
 - d. Protective clothing worn during procedures should be washed and disinfected at the end of each day.
7. Any new equipment or tools brought into the quarantine area must first be disinfected.

Seal Isolation

These measures should be followed if sick and healthy seals are housed at the same facility concurrently:

1. Use separate cleaning and feeding supplies, footwear and clothing exclusively for the sick seal unless instructed otherwise by the attending veterinarian.
2. Veterinary approval is required for any movements of seals between enclosures or when combining more than one seal in a tank.
3. If a seal requires isolation, follow the Potential Disease Outbreak Protocol.

II. OBSERVATIONS AND CONDUCT AROUND SEALS

A. OBSERVATIONS

1. In the morning and prior to each feed, conduct a thorough inspection of the seals and pens before proceeding with further activity. Following each feed or handling event, monitor the seals' behavior closely. Perform a final inspection before leaving for the day.
2. Throughout the day, use the cameras to observe each seal at least every 60 minutes. Observe and record the condition and activity level of the seal. Record the presence, color, consistency and amount of feces, urine, and spew (and the ID of the seal that produced it, if known). Look for any harmful debris in or around pens.
3. Note anything unusual in a seal's appearance (eye discharge or cloudiness, nasal discharge, bite wounds, etc.) and behavior (lethargic, unresponsive, stereotypic behaviors, etc.). Notify attending veterinarian and animal care manager immediately of any abnormal changes in a seal's health.
4. Succinctly record any observations on the Daily Observation Sheet, including the time and observer's initials. Frequently used acronyms: BAR = bright, alert, and responsive; QAR = quiet, alert, and responsive.

B. CONDUCT AROUND THE SEALS AT ALL TIMES

Every possible effort should be made to minimize the habituation of the seals by reducing human-seal interactions.

1. Do not enter enclosures unless absolutely necessary.
2. When in enclosures, **DO NOT MAKE PHYSICAL CONTACT WITH SEALS** unless necessary for procedures requiring handling. Minimize going into the enclosure and the amount of time you spend in the enclosure as much as possible.
3. Minimize talking and noise when working with or near the seals and the enclosure. Move slowly and avoid startling gestures.
4. Whenever possible, observers should remain as inconspicuous and unobtrusive as possible to observe seals' normal behaviors in captivity and minimize their stress in captivity.
5. Each person entering an enclosure with the seal should be carrying a herding board, which should be within arms-reach at all times.
6. Outside of feeding sessions seals may display undesirable behaviors. Record these observations and follow these instructions:
 - a. Approaching too closely or too rapidly
→ Use a herding board to keep the seal away
 - b. Mouthing hoses, brooms, or boots
→ Discourage this by preventing opportunities for seals to bite at these objects
 - c. Stereotypic behaviors (repetitive splashing, slapping at the walls of the enclosure, pattern swimming)
→ These are a sign of boredom and may be reduced by providing seals with approved environmental enrichment devices (EEDs). Objects such as marine debris that the seals may encounter once returned to their natural habitat should not be used as EEDs so that they do not associate these objects with food or play. A good example of an EED is sinking a milk crate that has fish stuck in the holes or providing some of their daily caloric needs through "fishsicles."

III. CLEANING THE QUARANTINE AREA

A. DISHES

1. Wash all dishes used for feeding and handling with dish soap and water. Scrub the inside of all feeding tubes using a tube brush. Rinse thoroughly.
2. Place all dishes in a dish sanitizer. If a dish sanitizer is not available, the following steps should be followed after step 1, above:
 - a. Soak or spray all equipment (bolus syringes, knives, tongs, cutting boards, etc.) with disinfectant according to section I.B. (“Approved Disinfecting Agents”) above.
 - b. Rinse all dishes thoroughly to remove the disinfectant.
 - c. Allow all dishes to air-dry.
 - d. Stomach tubes should be washed with soap and water, rinsed thoroughly, and then boiled for 10 minutes.
3. Bolus Syringe Care: after the syringes have been washed and dried as described above, lubricate the O-ring with mineral oil and put the syringes back together for safe storage. Be careful when handling the syringes as they are fragile and can crack easily.

B. DAILY CLEANING AND MAINTENANCE

Seal Enclosure Cleaning

1. Do not allow seals to mouth or bite brooms or hoses.
2. Never allow equipment to remain unattended in an occupied seal enclosure. Return all equipment to its storage area after use.
3. Always keep enclosure gates bolted.
4. When cleaning, take the opportunity to look for vomit, diarrhea and observe the feces for consistency and parasites. Always record observations form in the seal’s chart and make special note of any unusual findings.
5. Every morning, inspect the entire pen enclosure for any scat, urine, fish parts, and wind-blown debris. If necessary, use a broom and fresh water hose to clean the seal enclosure. Thoroughly rinse all fish scales, blood, and debris from the decks, walls, and ledge of the enclosure and walkway with the fresh water hose after each feed. Special care should be taken to clean scales from doors, door handles, and bolts.
6. Before leaving in the evening, the deck and pool walls and floor should be hosed down and any spattered blood, scales, scat, or other debris should be scrubbed away.

Footbaths and Walkways

1. Rinse off the walkway and stairs leading to the seal enclosure at least once a day. Scrub the walkway with broom, disinfectant and water as needed.
2. Refill footbaths as needed depending on choice of disinfectant (usually once per 1-2 days for Accel). When using bleach, footbaths should be refilled anytime organic material is present.
3. If using bleach, add 1 cup bleach to 1 gallon of water and be sure to have a final water rinse before the pen entrance.

Fish House Cleaning

1. Freezers and refrigerators must remain clean and neat at all times. All feeders are responsible for maintaining freezer cleanliness on a daily basis. Keep freezers free of ice buildup as much as possible.
2. Wipe down all counter and table surfaces after each feeding. Be especially mindful of cleaning any fish scales and spattered blood from the all surfaces after each feeding.
3. Mop the food prep room floor after the morning feeding.
4. Empty the garbage daily.

C. WEEKLY CLEANING

Seals should be crated/kenneled and weighed once weekly using the forklift. Weekly cleaning can be done during this time. Use a net to scoop the seals out of the water and herding boards to direct them into the holding area. Be sure to keep the seals wet, shaded and monitor their behavior regularly.

Seal Enclosure

The monk seal pools should be drained and the pools, walls, ledges, doors, and stairways cleaned once a week using accelerated hydrogen peroxide disinfectant (preferred) and a large, soft-bristled brush.

1. Drain pool, empty all footbaths.
2. Spray and use disinfectant to scrub all surfaces (pools, walls, ledges, doors, stairways).
 - a. If using bleach solution instead of hydrogen peroxide, all organic matter must be rinsed away first and be careful to direct the rinse water toward the drain holes at the corners of the enclosure, away from seals because (bleach is a skin and eye irritant).
3. Allow appropriate amount of contact time for the disinfectant used (see above).
4. Hose off all surfaces, then close drain and turn on the water inflow.
5. Refill footbaths and when pool is full, return seals to enclosure.
6. Thoroughly rinse and put away all cleaning equipment.
7. Record the seals' behavior, the duration spent in the holding area, and any other relevant information from the cleaning event (scat, spew, urine, etc.) on the observations form in each seal's chart.

D. QUARTERLY CLEANING

Every 3 months, and particularly before the rainy season or forecasted heavy rainfall, the shade structure should be rinsed (if removable, it should be removed and scrubbed) to remove dust and debris. Rinse water should not go into an enclosure if it is occupied by a seal – remove the seal as with weekly cleaning procedures. Clean enclosure per weekly cleaning instructions after cleaning the shade structure.

IV. WATER SAMPLING SEAL TANK

Sampling should occur on the same day and time each week at least a couple of days after the weekly enclosure cleaning. Collect one sample from each occupied pool and one from the inflow in addition to a temperature control sample collected from the pool. These samples are submitted to Hawaii Food & Water Testing Lab (HF&WTL) for total coliform testing.

1. Be as sterile as possible: wear gloves, do not open lid to bottle until immediately before collection, do not contaminate inside of lid or bottle, don't set the lid down, etc.
2. Collect the inflow sample by removing the lid and holding the bottle under the water inflow to fill it. Decant any excess water being careful not to touch the lip of the bottle or the lid.
3. Sample the pool (pool and temp control sample) 180° from the water inlet. With the lid still in place, submerge the bottle about 1 foot deep. Unscrew the lid underwater with the bottle positioned counter-current to fill the bottle. Replace the lid underwater. Remove the bottle from the water and decant the excess water being careful not to contaminate the bottle or lid.
4. Immediately place the samples in the small red cooler with blue ice (provided by HF&WTL) for transport to the lab. If transport is not immediate, place the samples in the refrigerator (sampling fridge, not fish storage fridge). Store sample bottles in the cooler and ice pack in freezer until next sampling.
5. Complete all the necessary paperwork and be sure to label each bottle (pool, inflow, temp control).
6. Results submitted on Tuesday are usually faxed to us, c/o Angie Kaufman, on Thursday or Friday. These counts should not exceed 1000 MF/100ml. If fecal coliform counts exceed 1000 MF/100ml, results are reported to Robert Dollar by phone; sampling must be repeated within 24 hours. Promptly notify the veterinary staff if counts are above 1000 MF/100ml.
7. Enter the date, time, coliform count, and any pertinent comments in the HMS Water Testing spreadsheet.

DIRECTIONS TO HF&WTL

2688 B Kilihau St.
Honolulu, HI 96819
Ph: 836-5558
Fax: 836-5509
contact: Wendy

Open Mon.-Friday, 8am-5pm

Located in Mapunapuna near the airport. Go east (towards the airport) on Nimitz Hwy & turn left on Kakoi St then right on Kilihau St (2688B Kilihau St.). It's the 3rd grey building on the left.

V. SEAL ILLNESS/EMERGENCY CARE

1. In case of an emergency or suspected illness, refer to the phone list and call the attending veterinarian or veterinary technician immediately to relate symptoms or circumstances of emergency or illness. Follow the emergency chain-of-command protocol.
2. A veterinarian or trained veterinary staff will perform any needed blood sampling.
3. A crash kit and emergency drugs will be kept at all facilities when seals are present. All other medical supplies for blood sampling, fluid and antibiotic administration, monk seal medications, and additional medical supplies are kept at the Vet Lab Ford Island.

EXAMPLE Physical Examination Form
Circle as appropriate

Body outline: Swelling, Wound, Change from previous day

If yes, describe: _____

Flippers: Normal use of all 4 flippers with full-range of motion, Favoring one flipper (describe _____), Lacerations, Swelling, Ulcers/sores, Signs of pain or discomfort

Discharges: Ears, Nares, Eyes, Umbilicus, Rectum, Vagina, Other

If yes, describe amount: _____ mL, Color: _____, Consistency: _____

Feces: Describe amount: _____ mL, Color: _____, Consistency: _____

Urine: Color: _____

Eyes:

Right: Discharge: Clear tears, Crustiness around eyes, Purulent discharge
Redness or congestion of conjunctiva, Swelling of conjunctiva, Prominence of third eyelid, Corneal opacity/ cloudiness, Corneal ulcer, Lacerations, Swelling of eyelids, Squinting or photosensitivity, Any obvious loss of vision

Left: Discharge: Clear tears, Crustiness around eyes, Purulent discharge
Redness or congestion of conjunctiva, Swelling of conjunctiva, Prominence of third eyelid, Corneal opacity/ cloudiness, Corneal ulcer, Lacerations, Swelling of eyelids, Squinting or photosensitivity, Any obvious loss of vision

Mouth: Color of mucous membranes: Pink, Red, Pale pink/White

Teeth: Broken, Erupting. List site: _____

Behavior: Alert, Bright, Lethargic, Depressed, Active, Inactive, Stereotypic behavior, Disorientation, Vocalizations, Other abnormal behavior for each individual seal, Any marked change from previous days

Describe: _____

Other comments (environmental conditions, respiration rate, heart rate, etc.):

Animal ID: _____ **Date:** _____ **Name of Observer:** _____

Time: _____

File No. 16632 Appendix G: Necropsy Protocols

The following are current necropsy protocols and forms. There are separate protocols and forms for the Northwestern Hawaiian Islands (NWHI) and main Hawaiian Islands (MHI).

NWHI HAWAIIAN MONK SEAL NECROPSY PROTOCOL

SAFETY CONSIDERATIONS

Before performing a necropsy, be sure you have read the following documents located in the **Zoonotic Disease** section of your **Master Field Log** and your camp's **Necropsy Manual**:

Occupational Safety: Working with Marine Mammals and Your Health
<http://www.vetmed.ucdavis.edu/whc/mmz/Occupational%20Safety.htm>

Marine Mammal Zoonotic Bacteria
<http://www.vetmed.ucdavis.edu/whc/mmz/bacteria.htm>

Marine Mammal Zoonotic Viruses
<http://www.vetmed.ucdavis.edu/whc/mmz/viruses.htm>

Zoonosis and Quarantine (MARP Manual)

Appendix II: Infectious Agents (Aguirre *et al.*, 1999)

Public Health (Cowan *et al.*, 2001)

Assessment of the Risk of Zoonotic Disease Transmission to Marine Mammal Workers and the Public: Survey of Occupational Risks*
http://swfsc.noaa.gov/uploadedFiles/Divisions/PRD/Programs/Photogrammetry/Marine_Mammal_Zoonoses_Final_Report-2.pdf

*Only available online and on your field computer:

Preventing Disease Transmission

Avoid direct contact with dead seals to prevent transmission of infectious diseases that may be pathogenic to humans.

Persons performing the necropsy must:

1. Cover all surface wounds with a protective dressing before gearing up.
2. Wear protective gear, including latex or vinyl gloves, mask, disposable gowns, and foot covers. Change torn gloves **immediately**.
3. Seek medical attention **immediately** if you get any cuts, punctures or other injuries during the necropsy. Notify the attending physician of the source of the injury.
4. Disposable items such as scalpel blades, needles and biopsy punches **MUST** be disposed of in sharps containers.
5. If possible, pull carcass up the beach to higher ground and bury it after necropsy to avoid attracting scavengers and to minimize the potential for disease transmission.
6. Disinfect all instruments and contaminated equipment after the necropsy has been performed (see Post Necropsy section below).
7. Once the necropsy has been performed and all gear has been cleaned and disinfected, wash thoroughly with soap. Disinfect reusable clothing with bleach solution (see tagging handling protocol) and dispose of all contaminated clothing, gloves, gowns, etc. in a biohazardous waste bag.
8. **DO NOT STORE ANY SPECIMENS IN FREEZERS/REFRIGERATORS USED FOR HUMAN FOOD.**

GENERAL CONSIDERATIONS

A necropsy is a systematic examination of the whole body, organs, and tissues and is a basic tool for investigating disease and for monitoring the health of the Hawaiian monk seal population. **Whenever possible, necropsies should be performed by a trained veterinary pathologist** experienced in recognizing and interpreting lesions and abnormalities.

Necropsy How-To Guides:

For general guidance on the steps in performing a necropsy, please refer to the following resources, but follow the sample collection protocols provided in this document and the most recent version of the Necropsy Report Form.

1. **Field Manual for Phocid Necropsies (specifically *Monachus schauinslandi*)** (FMPN) located in your camp's **Necropsy Manual**
2. **Marine Mammal Necropsy: An Introductory Guide for Stranding Responders and Field Biologists** located on your field computer and also available at: <http://www.bahamaswhales.org/strandings/necropsy.pdf>

Necropsies will have the most scientific value when they are carefully documented. Adherence to this protocol and the **Necropsy Report Form** will assist in the documentation and standardization of information, which may be valuable in determining morbidity and mortality factors within the population and as well as for individual seals.

Things to keep in mind:

1. Record all observations – when in doubt, just describe what you see.
2. The order of the **Necropsy Report Form** follows the sequence of general dissection and examination. If you are skilled and familiar with Hawaiian monk seal necropsies, you may find it easier to use the Necropsy Specimen Checklist, but **be sure to have someone record all observations, photos, measurements, and descriptions of organs on the Necropsy Report Form.**
3. Tissues and organs must be examined in a systematic manner. The precise method used for a necropsy is less important than establishing a routine in which each body system is examined fully.
4. **Once the carcass has been opened, take tissue specimens for virology, bacteriology and toxicology first, then sample for histopathology and parasitology.**
5. Samples of **normal and abnormal** tissue should be collected for laboratory analyses.

The ability to obtain reliable data from necropsies depends on the following:

1. Condition and location of the carcass
2. Adherence to detailed protocols
3. Number of seals necropsied throughout the year
4. Amount of time available to perform a thorough necropsy
5. Care in sample preservation and labeling of specimens
6. Care in shipping and storing specimens

Decomposed carcasses may be unsuitable for histopathology but can be useful for observing gross lesions. Collect brain samples regardless of the state of decomposition. Collect samples from all organs listed, even those that appear normal. In general, tissue specimens must be sufficiently thin (**less than 1 cm thick**) to allow proper fixing of 10 parts 10% buffered formalin: 1 part tissue. For some tissues (e.g. brain and lung), you may need to make parallel cuts (0.5 cm in thickness) in the tissues to allow preservation. After the tissues have been fixed in formalin 24-48 hours, pour off the formalin, rinse the tissues in fresh water, and add fresh formalin solution.

NECROPSY INSTRUCTIONS

Complete a **Hawaiian Monk Seal Necropsy Report Form** for **all** carcasses recovered. Use the **full form** if you perform an internal examination of the carcass, regardless of the condition code. The **partial form** can be used for necropsies where very minimal data is collected. Record "N/A" for any sections that are not applicable, and state what organs/tissues were not examined. At a minimum, describe each organ examined and sample as many organs as possible, prioritizing the following tissues: **brain, lung, liver, kidney, blubber.**

Photograph the exterior for ID (even if tagged), to document injuries or other unusual conditions, and to document body condition. Photograph the seal from all 4 sides (dorsal/ventral/left lateral/right lateral) and a close up of the hind flippers with tags. In addition, take close-ups and a wider view (to show perspective) of injuries and unusual conditions. If possible, include an index card in each frame that notes the following: Seal ID, Date, Size, Sex, and island and a ruler. Record photographs on the Necropsy Report Form.

External examination

Document any specific external lesions, abnormalities, or scar patterns. Examine, describe, and photograph any external lesions or injuries, the anogenital area, scars and other distinguishing characteristics.

Experience has shown that in cases where pinnipeds have drowned, there is often a complete absence of expected gross and histological findings. For this reason, it is imperative to look closely for external indications of entanglement. Findings may

include: bent or missing vibrissae, torn or missing nails, and cuts in and around the nares, mouth, and gums. Closely examine the tips of all extremities to look for line or net cuts. Linear marks on the pelage are also of interest. Photograph any suspected abnormalities with close up/macro images, followed by images that demonstrate the location(s) on the body of each close up image.

Carcass condition codes

Evaluate carcass condition (state of decomposition). Carcass condition is influenced by many factors including disease, body temperature, and environmental temperature. **Rigor mortis** (stiffening of the body following death) may serve as an indicator of carcass evaluation. It can occur within hours in warm weather, but is extremely variable. *Rigor mortis* indicates that a carcass may be in good condition (Code 2).

Code 1: just died (*e.g.*, euthanasia)

Code 2: fresh/carcass in good condition (rigor mortis, fresh smell, normal appearance, minimal drying of skin and mucous membranes, eyes clear, carcass not bloated, muscles and blubber firm, viscera intact and well-defined, guts with no gas). NOTE: Rigor mortis (stiffening of the body following death) may serve as an indicator of carcass evaluation. It can occur within hours in warm weather, but is extremely variable. Rigor mortis indicates that a carcass may be in good condition

Code 3: fair/decomposed (carcass and organs intact, bloating, skin sloughing, mild odor, eyes sunken, dried mucous membranes, friable viscera, blubber oily, muscles soft but still intact, gut dilated with gas)

Code 4: poor/advanced decomposition (carcass may be intact but collapsed, skin sloughing, strong odor, blubber soft with pockets of gas, liquified organs, blood thin and black, viscera friable difficult to dissect and easily torn, gut filled with gas)

Code 5: mummified/skeletal remains (skin draped around bones, remaining tissues desiccated)

Tags

If flipper tags are present, note their condition on the survey form (data type 'T') and tag condition forms. Collect and place them in a whirlpak bag labeled with animal ID, island/atoll, date, and survival factor number. Scan the entire body for PIT tags by holding the PIT tag reader as close to the body as possible. Even if PIT tags are not found, indicate on the survey sheet that a scan was completed and where on the body the scan was performed.

Morphometric measurements

Axillary girth – At the armpit, measure the circumference around the entire body in centimeters.

Standard length – Measure the straight line (not curved) length of the entire seal from the tip of the nose to the tip of the tail in centimeters. If a scale is available, weigh the body and report units. **Record measurements on both the TAGGING/HANDLING CARD and the Necropsy Report Form.**

Swab Collection

Use sterile Dacron swabs. Avoid touching swab tip to anything other than the tissue being swabbed. Immediately place swab in cryovial and break off the end of the plastic applicator against the side of the cryovial container (it should snap easily).

Internal Examination

TAKE INTERNAL PHOTOGRAPHS ONLY WHEN UNUSUAL CONDITIONS ARE NOTED OR

IF YOU ARE UNSURE IF IT IS UNUSUAL. If unusual conditions are noted, include a size reference (*e.g.*, ruler) and label with seal ID, survival factor number, date, and island. Take two photographs, one with the organ *in situ* (in its anatomical position/location) in the body and one with the organ removed from the body and placed on a solid white or light blue surface.

Record complete and thorough observations. Assume more is better when describing and recording information. The rule here is if in doubt, write it down. If unsure whether something is abnormal, state this and succinctly describe. Descriptions should be clear, concise, and without personal interpretation. Appropriate tissue preservation along with YOUR precise description of findings may allow the identification of causes of death in the population.

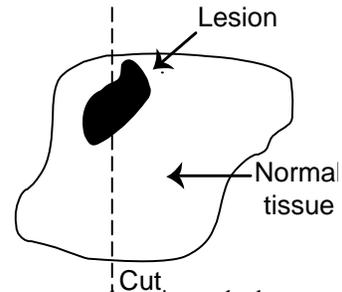
Identify the appropriate descriptors for each organ examined. The descriptions provided herein are NOT an exhaustive list of terms, but rather a list for your reference. **Describe surface, consistency, color, and cut surface of both normal tissues and abnormalities or lesions.**

Descriptors of Organs and Lesions

- Surface: Smooth, rough, shiny, dull, thickened, wrinkled.
- Consistency: Firm, soft, flabby, dry, wet, fluid-filled, sharp-edged, friable (easily pulverized or crumbled).
- Color: Transparent, translucent, opaque; white, cream, green, yellow, brown, pink, red, nutmeg (normal pattern of liver), etc. Use simple colors, do not get complicated. Also comment how color is spread through tissue- homogeneous, speckled, streaked, blotchy, blanched, mottled (i.e., pink with specks of red). Additional descriptors may include bright, pale, dark.
- Cut surface: Slice organ several times appropriately and spread apart to look at internal surface. Be sure to describe color of the cut surface. Descriptors include swollen, bulging, shiny, dull, eroded, glistening, scaly, pitted, oozing
- Size: Record in metric system (mm, cm), measure length, width and depth or diameter of the lesion. Enlarged, (hypertrophied), small (atrophied), normal size.
- Shape: Square, rectangular, triangular, oval, round, cuboidal, spherical, discoid, rhomboid, tear-shaped, wedge-shaped, spindle-shaped, irregular, long, slender, indented, narrow, lace-like, tortuous, branching, speckled (miliary), flat, raised, depressed, shrunken, papillary, cauliflower-like.
- Distribution: Single discrete lesion (focal), multiple lesions in one location (multifocal), or multiple lesions scattered diffusely throughout the organ or body cavity (diffuse); locally extensive, random, even.
- Location: Surface, capsule, wall, dorsal or ventral, caudal or cranial, anterior or posterior, medial or lateral, proximal or distal, internal or external, full or partial thickness of a wall of an organ.
- Fluid: Clear, cloudy, turbid, thick, thin, bloody, mucoid, exudate, dark, tarry
- Consistency: Spongy, granular, gel-like, firm, soft, hard, rock-hard, dense, creamy, buttery, brittle, lumpy, velvety, warty, tenacious, gritty.
- Cut surface: Bulging, engorged, granular, nodular, pitted, oozing
- Odor: None, sweet, sour, rancid, ammonia-like, putrid, fruity, petroleum- like

Collecting necropsy tissues

Each complete necropsy should have two jars containing complete tissue sets of all tissues, and both having the same specimen number. One set should be sub# A and the other sub# B. Tissue set A should be the most complete set, (e.g., if you freeze one eye, tissue set A should have the formalin fixed eye). If there are any unusual lesions in any of the tissues sampled, be sure to include the margin between abnormal and normal tissue in both tissue sets A and B.



Collect samples of ALL LESIONS in formalin. Describe and sample areas that appear to stand out in marked contrast to the main body of tissue. Samples should include the margins between the normal and abnormal tissue and a description (i.e., sharp line versus vague and gradual, circumscribed, encapsulated). Make sure to check the boxes next to the appropriate specimens as collected on the necropsy report form.

Tissues for Toxicology (contaminants and biotoxins): Code 1, 2 ideal. Codes 3, 4, 5 useless.

Toxicological analyses may be performed for heavy metals, organochlorides, selenium, and dioxin. When sampling for toxicology, it is important to use standardized sampling procedures so that even when low levels of contaminants are present, differences may be attributed to biological processes and contaminant exposure and not to variation in the collection process.

For MHI necropsies, follow NIST sample collection protocol to collect toxicology specimens (this has been incorporated into the MHI necropsy form).

Tissues for Microbiology: Code 1 ideal; Codes 2, 3 limited; Codes 4, 5 useless.

Collect by special request only. Specimen collection for bacteriology and virology is determined primarily by the nature of gross pathologic lesions. Samples should be taken aseptically, from external surfaces, body cavities and internal organs as soon as they are exposed. Place swabs in respective transport media and refrigerate at 4 C or place on blue ice immediately and freeze upon arrival to laboratory or field camp. If cryovials are available, ultrafreeze the swabs with tissue samples in liquid nitrogen. Samples for microbiology are worth the time and effort only when tissues are in suitable condition. With an

"aborted fetus", perinatal death, or newborn in main Hawaiian Islands (MHI), collect specimens according to "Fetus" section of MHI Necropsy Form and refrigerate for microbial analysis.

Post Necropsy

1. Review the completed **Necropsy Report Form**, making sure that all boxes have been checked off on the form for all samples collected.
2. Necropsy Report Forms, photos, "List of Specimens Collected", and any other pertinent data should be returned to the NMFS PIFSC Honolulu Laboratory.
3. Refer to the section "Preventing Disease Transmission" #4-8 on page one for post-necropsy clean-up tips. Clean necropsy tools (you may also need to spray them with WD-40 or LPS) and restock necropsy kit so that it is ready for the next necropsy.
4. Change the formalin for all formalin fixed tissues as noted above.
5. Make sure that the tagging/handling card, scar card, and tag condition drawing form are complete. **Necropsy Report Forms**, scar cards, tagging/handling cards, survival factor forms, and photos should be returned to the NMFS Honolulu Laboratory.
6. Record specimens collected on the **Specimen Collection Summary** and assign specimen numbers as outlined in the **Specimen Collection Protocol**.
7. Clean necropsy tools. Before disinfecting, remove all organic matter from instruments by washing them thoroughly with warm (if possible) soapy water. If instruments are not cleaned properly before disinfecting, the remaining organic matter may shield organisms from destruction, and may inactivate the disinfectant. Be sure to wear proper protective gear (gloves, masks, etc.) when washing instruments. To minimize aerosolization, keep instruments below the water line when washing. Disinfect instruments with 70% alcohol or a 1:10 chlorine bleach solution for at least 10 minutes. However, bleach corrodes stainless steel, and may pit the instruments. Regardless of disinfectant used, be sure to thoroughly rinse instruments with fresh water after disinfecting. Air dry all instruments thoroughly before putting them away. You may also need to spray them with WD-40 (or LPS)
8. Restock necropsy kit so that it is ready for the next necropsy.

HAWAIIAN MONK SEAL NECROPSY REPORT FORM (NWHI)

SEAL ID/temp ID _____ DEATH/NEC. # _____ (assign sequential #'s by calendar date for all seals)
Date/time: necropsied _____ found dead _____ last seen alive _____
Island/Atoll _____ Islet _____ Sector _____ Lat _____ Long _____
Beach position _____ Carcass orientation (i.e., horizontal to water line) _____
Size/sex _____ Age (if fetus, refer to pg. 10) _____
Persons performing/assisting with necropsy: _____
RECORDER: _____ PHOTOGRAPHER: _____
Photos? Y / N File names: _____

HISTORY

Identifying body markings (take photos): _____
Last live observation(s): _____
Circumstances of death (found dead/euthanized/other-explain): _____

Tags

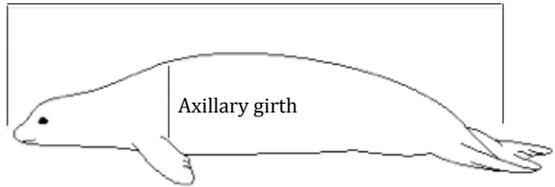
Record number and condition of any flipper tag(s), photograph, and collect all tags in labeled whirlpak.
R _____ L _____ Notes: _____

Scan the entire body for pit tags. PIT tag#(s):
R _____ L _____ Location found _____

Morphometrics

Axillary girth _____ (cm)
Straight length _____ (cm, circle: DSL or VSL)
Total body mass _____ (circle: kg/lb)
Measurer _____

Straight Length (tip of nose to tip of tail)



Carcass Condition Code: 1 2 3 4 5

- Code 1: Just died (e.g., euthanasia)
Code 2: Fresh, good condition (rigor mortis, fresh smell, normal appearance, minimal drying of skin/mucous membranes, eyes clear, carcass not bloated, muscles and blubber firm, viscera intact and well-defined, guts with no gas).
Code 3: Fair/decomposed (carcass and organs intact, bloating, skin sloughing, mild odor, eyes sunken, dried mucous membranes, friable viscera, blubber oily, muscles soft but still intact, gut dilated with gas)
Code 4: Poor/advanced decomposition (carcass collapsed, skin sloughing, strong odor, blubber soft w/ pockets of gas, liquified organs, blood thin and black, viscera friable difficult to dissect and easily torn, gut filled with gas)
Code 5: mummified/skeletal remains (skin draped around bones, remaining tissues desiccated)

GROSS NECROPSY EXAMINATION

INSTRUCTIONS

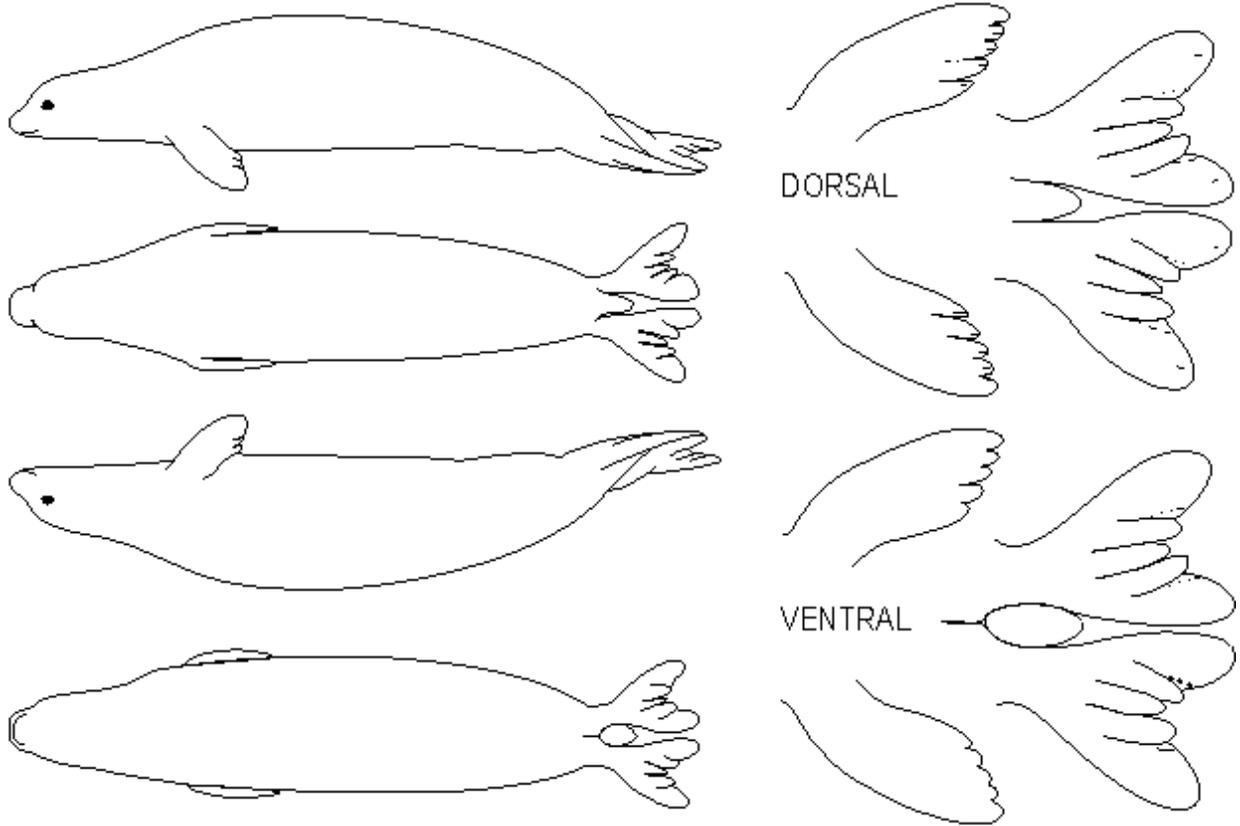
- 1. Describe all organs, tissues and lesions in the fields provided. If appropriate, circle either NSF, NE or NA at the top of each section. If the tissue examined is abnormal, describe the location, color, size and distribution within the tissue in the blanks provided.
2. Formalin fixed tissues should be no bigger than 2x2x1cm.
3. Collect 2 equal Tissue Sets (set "A" and "B") from all organs, to be fixed in formalin.

Tissue Set A = Sp # _____ A Tissue Set B = Sp # _____ B

- 4. If possible, remove head and place in cooler on ice while remainder of necropsy is completed, then go back and sample brain after the remainder of the necropsy is complete.
5. Examine all outside surfaces and note abnormalities, discharge, etc. Describe/draw abnormalities below. Photograph with full-frame views of all 4 sides (dorsal/ventral/left lateral/right lateral) as well as close-ups of scars or other distinguishing characteristics, injuries, abscesses, line marks or other abnormalities. Include an index card in the image frame that notes the following: Seal ID, Date, Size, Sex, SF#, Necropsy #, Location (island/atoll).

NOTE: In cases of drowning, there are few clues, if any, so look closely for external indications of entanglement (bent/missing vibrissae, torn/missing nails, cuts in and around the nose, mouth, and gums). Also examine the tips of all extremities to look for line or net marks. Describe/draw and photograph any evidence of entanglement. Collect any gear found on an animal in a ziploc bag and label.

Draw all abnormalities and markings (include bruises, wounds, contusions, old scars, condition of and tears of skin, external parasites):



ABBREVIATIONS

NSF: no significant finding

DB: DMSO/blue ice

NE: not examined

DR: dry

EXTERNAL PHYSICAL EXAMINATION (Circle all that apply):

GAS/ PUTREFACTION: (Run your hands firmly along the body and feel for bubbles.) NSF / NE / NA

NUTRITIONAL STATE:

Fat/overweight, Normal/average, Thin/poor, Starving/emaciated

Notes: _____

DISCHARGE: NSF / NE / NA

Location: eyes, nose, mouth, genitals, anus Color/Texture: green, white, clear, red, thick, runny

Notes: _____

SWAB COLLECTION: NSF / NE / NA

Use sterile Dacron swabs. Obtain 2 swabs each from: eyes (ES), nasal cavity (NS), oral cavity (OR), genital opening (GS), rectum (RS). Both swabs from the same orifice can be placed into the same cryovial, unless the eyes are abnormal (i.e., you should have a total of five 2.0 ml cryovials with 2 swabs from each orifice in each). However, if one or both eyes are abnormal, store the swabs separately and indicate below which vial contains the abnormal eye.

Sp # _____ Eye swab (LN) Sp # _____ Genital swab (LN) Sp # _____ Oral swab (LN)

Sp # _____ Nasal swab (LN) Sp # _____ Rectal swab (LN)

EYES: NSF / NE / NA

NSF, NE, missing, bulging, deflated, out of socket, foreign body present, penetrating wound

Notes: _____

Aqueous humor: collect using sterile needle and 3cc syringe. If both eyes are normal, aqueous can be combined in one cryovial. If one or both eyes are abnormal, use a clean needle and syringe to collect each sample and place in separate cryovials. Indicate which eye is abnormal above.

Sp # _____ Aqueous humor (LN)

Eyes: Collect both eyes. Fix one eye in Tissue Set A and freeze the other eye in a whirlpak. If an eye is abnormal, it should be fixed rather than frozen. If both eyes are abnormal, fix one in each Tissue Set. To fix an eye, make a 2-3 cm cut in the globe along the interface of the sclera (white part) and the clear cornea at the front of the eye before placing in formalin.

Eye (FM) A **AND** Sp # _____ Eye (LN) **OR** Eye (FM) B

MUCOUS MEMBRANES: NSF / NE / NA

Pink, pale pink, red, yellow, white, purple, brown, other: _____

ORAL CAVITY: NSF / NE / NA

Ulcers, vomitus, blood, foreign body, other: _____

TEETH: NSF / NE / NA

Unerupted, just erupting, fully erupted, missing, broken, worn (describe): _____

LEFT (top/bottom): # incisors ___/___, # canines ___/___, # post-canines ___/___

RIGHT (top/bottom): # incisors ___/___, # canines ___/___, # post-canines ___/___

VIBRISSAE: NSF / NE / NA

Absent, torn, other: _____

Collect two vibrissae (with roots) and freeze in a cryovial or whirlpak Sp# _____ Vibrissae (LN)

NAILS: NSF / NE / NA

Absent, torn, bleeding, cracked, crushed, other: _____

PERIPHERAL LYMPH NODES: **NSF / NE / NA**

Feel around the point of the shoulder (prescapular LN), and the angle of the jaw (mandibular LN)
Palpable, obvious, unnoticeable, other: _____

HAIR COAT: **NSF / NE / NA**

Hair missing, oil, molting, scruffy, parasites, foreign bodies, fishhooks, scavenging, abrasion, other:

SKIN: **NSF / NE / NA**

Cracking, bleeding, dry, moist, smooth, rough, wounds, masses, vesicles, ulcers, scars, bruising, abscesses, masses, abnormal coloration, other: _____

Collect two 1x1cm flipper tips in a 2mL cryovial pre-filled with 1.8 ml DMSO then freeze. **For the proper ratio of DMSO to tissue, please ensure that samples approximate the size of a pencil eraser.

Sp# _____ A TP (DMSO/LN)

Collect two samples from any masses, ulcers, vesicles or other external abnormalities. Fix one sample each in Tissue Set A and B(if possible collect unusual findings for both sets A and B, if this is not possible, unusual findings should go into at least set A) and freeze the other in a whirlpak. Try to include the junction of normal and abnormal tissue in the sample. Describe lesions sampled: _____

Skin lesion(s) (FM)A (FM)B Sp# _____ Skin lesion(s) (LN)

EXTERNAL GENITALIA & ANUS: **NSF / NE / NA**

Swollen, protruding/prolapsed, diarrhea, ulcerations, masses, plaques, other: _____

Collect and fix anything appearing abnormal. Abnormal genital tissue (FM)A (FM)B

MAMMARY GLANDS: **NSF / NE / NA**

Lactating, swelling, discharge, parasites, other: _____

If lactating, collect as much milk as possible and freeze. Sp # _____ Milk (LN)

OTHER NOTES ON EXTERNAL EXAMINATION:

INTERNAL EXAMINATION:

BLOOD **NSF / NE / NA**

NOTE: Unused PAX gene tubes should be stored at room temperature and not exposed to excessive heat or cold before use.

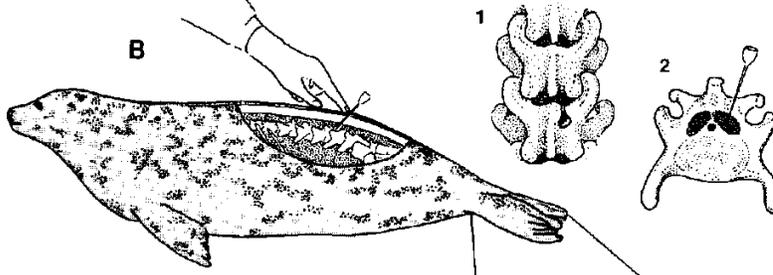
For code 1 carcasses only, attempt to collect blood (1 to 2.5ml) from the epidural venous sinus into a PAX gene tube (see below). Gently rock tube to mix blood and additive. After blood collection, store tube UPRIGHT at ROOM TEMPERATURE for a minimum of 2 hours (longer is ok, just no more than 72 hours). After the 2 hour incubation, transfer sample to cryovial(s) using disposable pipette and place on blue ice (or -20C freezer if available) for 24 hours. After 24 hours on blue ice, transfer cryovials to dewar. If no blue ice is immediately available, tubes may be stored in a refrigerator for up to 5 days before being transferred to blue ice and then to the dewar. Avoid placing samples directly from room temperature storage into the dewar, as this will likely ruin the sample.

Palpate the vertebral column and pelvis and move your fingers cranially 2 or more vertebral spaces, feeling for a “divot” lateral to the spinous processes of the vertebrae. Attach a needle to a 3cc syringe.

Needle choice:

- Pups/weaners: use a 20g or 21g x 1 1/2" needle.
- Adults: use a 3.5" spinal needle. Before insertion, remove the stylet, holding needle from hub only.

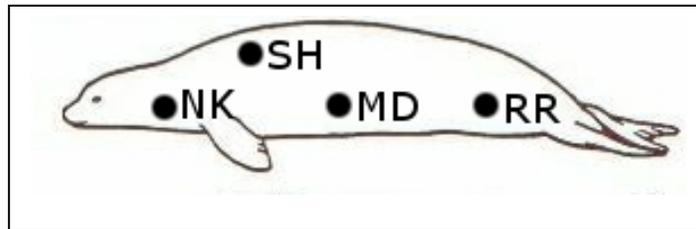
The angle of the needle may vary from a 45 - 90 degree angle to the dorsal surface of the animal. As the needle is inserted, feel it moving through skin, blubber, and muscle until you feel it pop through the membrane of the extradural sinus. Draw back on syringe to collect blood. Put a fresh needle (a small one is fine) on the syringe and push needle through top of PAX gene tube. The vacuum in the syringe should draw the blood into the tube.



BLUBBER

NSF / NE / NA

Thickness (measure on midline of chest between the front flippers): _____ mm



Samples from RR location (either side of the body is ok):

Code 1 and 2 animals only:

Use a 6 mm biopsy punch. Collect **8 blubber samples** through the **full depth** of the blubber layer, about 2-5 cm, until you reach the muscle layer. Use thumb forceps and/or scissors to retrieve the samples, without damaging or contaminating the blubber tissue. **One punch can be used to collect all 8 samples.** Take great care to prevent contamination of the samples by contact with gloves or other items. **The 4 best full thickness biopsies should go into the 5.0 ml cryovial, and the other 4 should go into the Teflon vial.**

Sp# _____ BB/FA from RR x4 (blubber for fatty acid in cryovial) (LN)

Sp# _____ BB/TX from RR x4 (blubber for toxicology in small Teflon vial) (LN)

All animals:

Collect two 1x1x1cm samples of skin and blubber from the RR location and fix one in each Tissue Set.

Skin/Blubber (FM) A (FM) B

Samples from NK, MD and SH locations (either side of the body is ok):

Collect one **full thickness**, 2 x 2 cm blubber sample from each of the following locations: neck (NK), shoulder (SH) and midsection (MD) locations. Place each sample in separate whirlpak (do not wrap in foil), and label each bag with the body site from which it was collected.

Sp# _____ BL/NK one 2 x 2 cm from NK (fatty acids) (LN)

Sp# _____ BL/MD one 2 x 2 cm from MD (fatty acids) (LN)

Sp# _____ BL/SH one 2 x 2 cm from SH (fatty acids) (LN)

CEREBRAL SPINAL FLUID (CSF)

NSF / NE / NA

Cut through the skin, blubber, nuchal ligament, and neck muscles to the dorsal aspect of the atlantoccipital skull joint (trying to avoid cutting into joint). Slowly insert needle w/ syringe approximately 5-7 mm into the foramen magnum. Collect up to 3mL into a cryovial.

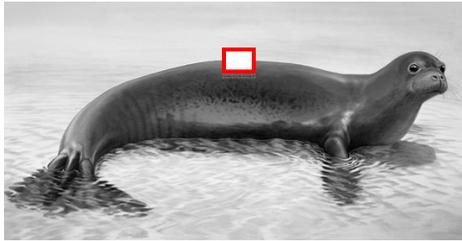
Sp # _____ CSF (LN)

MUSCLE

NSF / NE / NA

Describe abnormalities: _____

Collect the following samples from the middle of the the *longissimus dorsi*, the large long muscle that runs down either side of the spine:



One 2x2x2 inch muscle sample for myoglobin analysis. **Double bag** the sample in whirlpaks and freeze.

Sp# _____ A MU (LN)

One 1x1x0.5cm piece for stable isotope analysis and freeze.

Sp# _____ B MU (LN)

Two 1x1x1cm pieces in formalin and place one in each Tissue Set.

Muscle (FM) A (FM) B

ORO/NASOPHARYNX

NSF / NE / NA

NASOPHARYNX: Look for nasal mites. If observed, please approximate quantity: <10 10-30 30-50 >50

Fluid (Y / N) volume: _____ ml. Describe fluid: _____

TONSILS: enlarged, red, purple, pus, other: _____

Ulcers on tongue, ulcers on gums, ulcers on hard palate, foreign body, vomitus (volume: _____ ml, appearance: _____)

THORACIC CAVITY

Open the chest. Examine external surfaces of lungs and heart *in situ* and note abnormalities in appropriate section. Look for free fluid in the thoracic cavity (around the lungs, pooled at the diaphragm), collect 1-3ml if abnormal (see below) and describe.

Fluid present (Y / N), volume: _____ ml, appearance: purulent (thick/cloudy), serous, fibrinous, yellow, white, green, blood-tinged, frank blood, adhesions, plaques, other: _____

Sp # _____ chest fluid (LN)

Sp # _____ parasites (LN)

SALIVARY GLANDS: NSF / NE abnormalities: _____

THYROID: NSF / NE abnormalities: _____

LARYNX: NSF / NE abnormalities: _____

ESOPHAGUS: NSF / NE dilated, constricted, perforated, ulcerated, hemorrhagic, foreign body, fluid (volume: _____ ml, appearance: _____), other: _____

TRACHEA: NSF / NE perforated, lacerated, foam (mild / moderate / heavy), fluid (mucoïd / purulent / white / yellow / green / blood), volume: _____ ml. Mucosa: congested, hemorrhagic, ulcerated Notes: _____

BRONCHI: NSF / NE perforated, lacerated, foam (mild / moderate / heavy), fluid (mucoïd / purulent / white / yellow / green / blood), volume: _____ ml. Mucosa: congested, hemorrhagic, ulcerated Notes: _____

PARASITES: nematodes, other: _____ location: _____ Severity: <10, 10-20, 20-50, >50

THYMUS: NSF / NE atrophy, prominent, enlarged, other: _____

Note: the thymus shrinks with age, and is not likely be found on adult animals.

Submandibular LN (FM) A (FM) B Thymus (FM) A (FM) B

Tonsil (FM) A (FM) B Esophagus (FM) A (FM) B

Tongue (FM) A (FM) B Trachea (FM) A (FM) B

Thyroid (FM) A (FM) B Bronchus (FM) A (FM) B

BRACHIAL PLEXUS

NSF / NE / NA

If possible, collect the adjacent axillary LN with the brachial plexus and associated vessels and place in formalin.

Notes: _____

Brachial plexus (FM) A (FM) B

CRANIAL and THORACIC LYMPH NODES

NSF / NE / NA

PULMONARY LYMPH NODES: Look around the base of the heart (they are located at the largest end of the blood vessels going to the lungs). Collect paired samples in formalin and freeze one for microbiology.

Pulmonary LN (FM) A (FM) B Sp # _____ PN (LN)

MEDIASTINAL LYMPH NODES: Look around the heart and between the lungs. It is very difficult to determine the specific name of the node but all we are after are any abnormal lymph nodes and a few normal from the chest cavity. Collect paired sampled in formalin and freeze one for microbiology.

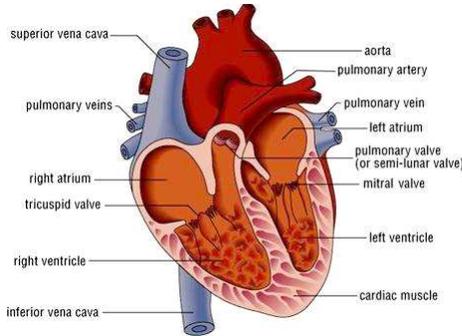
Mediastinal LN (FM) A (FM) B Sp # _____ MN (LN)

OTHER LYMPH NODES: Examine as many additional cranial lymph nodes as possible: mandibular, axillary, prescapular, sternal. Describe any abnormalities below. Suggested descriptors: hemorrhagic, gelatinous, serous fluid, soft, hard, enlarged (mild/mod/severe).

Collect 1x1x1cm samples from abnormal nodes and preferably 2-5 normal nodes, label with a laundry tag and fix one sample from each node in each Tissue Set. Be sure to include abnormal and normal tissue in the fixed samples. For code 2+ carcasses, sample the freshest lymph nodes, label and place one sample from each node in each Tissue Set.

List lymph nodes collected in formalin: _____ (FM) A (FM) B

HEART



PERICARDIUM (heart sac): NSF / NE thickened, plaques on surface, contains fluid (volume: _____ ml, describe: _____) Collect pericardial fluid if abnormal (>1 or 2 mL):

Sp # _____ HF pericardial fluid (LN)

PULMONARY ARTERIES AND AORTA: NSF / NE thrombi, plaques, rupture, other: _____

VALVES*: NSF / NE diffusely thickened, nodular thickening, vegetative/proliferative lesion (valve(s): _____)

**If valves appear abnormal, place a sample in each Tissue Set.*

LEFT / RIGHT VENTRICLES: NSF / NE thickened, dilated (location _____ thickness: _____ mm)

MYOCARDIUM (heart muscle): NSF / NE pale, tumors, abscess, white foci (location: _____)

ATRIA AND AURICLES: NSF / NE thickened, dilated, pale, tumor, abscess, white foci

Parasites describe: _____

severity: <10, 10-20, 20-50, >50

Notes: _____

Collect the following and fix one of each in each Tissue Set:

- Section through L. ventricle/Intraventricular septum/R. ventricle* (FM) A (FM) B
- Aorta (FM) A (FM) B
- Pulmonary arteries (FM) A (FM) B
- Section through R. atrium and atrioventricular valve** (FM) A (FM) B
- Section through L. atrium and atrioventricular valve** (FM) A (FM) B

*The intraventricular septum separates the right and left ventricles. The left ventricle is typically thicker-walled than the right ventricle.

**The atrioventricular valves are the valves located between the atrium and the ventricle on each side of the heart (left side = bicuspid; right side = tricuspid).

Next, collect (2) 4x4cm samples of heart tissue, wrap in foil, and freeze.

Sp # _____ A Heart (LN) Sp # _____ parasites (LN)

B Heart (LN)

LUNGS

NSF / NE / NA

Describe: pink, red, purple, mottled, congested, consolidated, abscesses, granulomas, emphysema, masses, interstitial edema

Specify location, distribution, severity: _____

Parasites: none detected 1 2+ 3+ 4+ Describe color, size: _____

Collect two 2x2x1cm pieces of lung and fix one in each Tissue Set. Collect additional samples if abnormalities are observed. Be sure to include both normal and abnormal tissue and describe abnormalities.

Lung (FM) A (FM) B

Collect (2) 4x4cm pieces of lung, wrap in foil, and chill on blue ice.

Sp # _____ A Lung (LN)
B Lung (LN)

ABDOMINAL CAVITY **NSF / NE / NA**

Open the abdomen and look for any excess or thickened fluid, collect up to 3ml of fluid in a cryovial and describe it below. Examine external surfaces of abdominal organs *in situ* (before you move them) and note abnormalities in appropriate organ section below. Fluid present (Y / N), volume: _____ ml, appearance: pus, serous (like serum), stringy, yellow, white, green, blood-tinged, blood, fibrous adhesions/scars, plaques, other: _____

Sp # _____ abdominal fluid (LN)

DIAPHRAGM **NSF / NE / NA**

Collect two 2x2 cm samples of the diaphragm and fix one in each Tissue Set. Notes: _____

Diaphragm (FM) A (FM) B

ADRENAL GLAND

Right: NSF / NE enlarged, shrunken, hemorrhagic, abscessed, dark, pale, other: _____

Left: NSF / NE enlarged, shrunken, hemorrhagic, abscessed, dark, pale, other: _____

Region (cortex vs medulla) and distribution of lesions: _____

Adrenal gland (FM) A (FM) B Sp # _____ Adrenal (LN)

GALL BLADDER **NSF / NE / NA**

Describe: full, empty, thickened wall, flukes (severity: <10, 10-20, 20-50, >50), other: _____

Bile: thick/chunky, thin/runny, black, dark green, light green, yellow, orange, stones present

If present, collect 1-3ml of bile (code 1-2 carcasses only) and place in a cryovial and whirlpak, wrap in foil to protect from light.

Sp # _____ Bile (LN)

Collect two 2x2x1 cm sections of gall bladder and fix one in each Tissue Set.

Gall bladder (FM) A (FM) B

LIVER **NSF / NE / NA**

Describe: enlarged, small, tan, brown, black, yellow, orange, mottled, abscesses, granulomas, masses, cysts, hemorrhage, parasites, other: _____

Collect at least two 2x2x1 cm of liver and fix one in each Tissue Set. Be sure to also include both normal and abnormal liver in each Tissue Set.

Liver (FM) A (FM) B

Collect and freeze: (3) 4x4cm liver samples, two 1x1x1cm samples and one 2x2x1cm sample and freeze.

Sp # _____ A liver 4x4cm (tox) (LN) D liver 1x1x1 (micro) (LN)

B liver 4x4cm (tox) (LN) E liver 1x1x1 (micro) (LN)

C liver 4x4cm (biotox) (LN) F liver 2x2x1 (stable isotopes) (LN)

Collect the hepatic lymph nodes for microbiology, chill on blue ice. Look around the base of the large blood vessel coming from the aorta nearest the liver.

Sp # _____ hepatic lymph nodes (LN)

PANCREAS **NSF / NE / NA**

Loss of lobulation, swollen, hemorrhage, abscesses, other: _____

Collect two 2x2x1 cm pices of pancreas and fix one in each Tissue Set.

Pancreas (FM) A (FM) B

SPLEEN **NSF / NE / NA**

Masses, enlarged (mild / moderate / severe), constricted, congested, abscesses, scars, pale, purple, brown, red, other: _____

Collect tissues for 2 sets for histopathology and one for microbiology (1x1x1 cm):

Spleen (FM) A (FM) B Sp# _____ 1x1x1cm spleen A (LN)

STOMACH **NSF / NE / NA**

Erosions, ulcers, perforated ulcers, hemorrhage, loss of rugal folds, swollen rugal folds, other: _____

Mucosa: white, pale pink, red, purple, other: _____

Parasites (ascarids): <10 10-20 20-50 >50 Describe (size, color): _____

Collect representative sample of parasites and freeze. Parasites Sp# _____ (LN)

Stomach contents: empty, dilated with gas, milk, mucus, fish (digested / partially digested / undigested), foreign body, other: _____

Collect stomach contents into whirlpaks or cryovials and chill on blue ice. Stomach contents Sp# _____ (LN)

Collect two 2x2x1 cm sections of stomach tissue and fix one in each Tissue Set. Be sure to also include any abnormal tissue (ulcers, thickened rugal folds, etc.).

Stomach (FM) A (FM) B

INTESTINES **NSF / NE / NA**

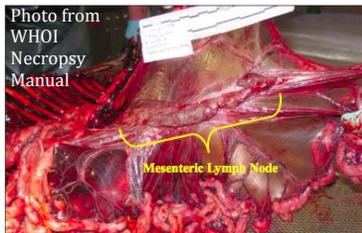
Open up ~4-6" of each section of the gastrointestinal tract, and look for abnormalities in color or thickness. Be sure to include any abnormal tissue as well as normal tissue in the formalin fixed samples. For intestinal samples, take a complete transverse "ring" of tissue ~1-2 cm in width, trying not to touch or disrupt the inside of the ring.

Duodenum (FM) A (FM) B Cecum (FM) A (FM) B

Jejunum (FM) A (FM) B Colon (FM) A (FM) B

Also collect one sample (2 cubic inches) of small intestine for microbiology and freeze. Small intestine Sp# _____ A (LN)

MESENTERIC LYMPH NODE:



Collect samples from the mesenteric lymph node anywhere along the intestinal mesentery, preferably both at the cranial and caudal portions of the abdomen/intestinal tract. Collect 2 sets for tissue set A and B, and freeze one whole for microbiology. Chill on blue ice.

Mesenteric Lymph Node (FM) A (FM) B

Sp # _____ ML (LN)

FECES: Cut colon near anus and squeeze contents from distal intestines directly into container.

FE (feces) Sp# _____

A. Collect sub-sample in pre-filled DMSO vial, fill to the 5 ml line on the vial

Feces A (DMSO)

B. Collect 1-3 g in a whirlpak for ciguatera analysis (FE/CX)

Feces B (whirlpak)

C. Freeze an additional 10-30 g in whirlpaks or wide-mouth cryovials for hormonal studies (FE/HR) (whirlpak/cryo)

Feces C

D. Freeze 10 g of feces (or meconium if newborn pup) into whirlpaks or cryovials

Feces D (whirlpak/cryo)

URINARY TRACT **NSF / NE / NA**

KIDNEYS:

Right: normal, congested, hemorrhage, abscess, parasites, cysts, hydronephrosis (distended), mass, calculi, emboli, infarct, loss of renule differentiation, other: _____

Size: normal, small, enlarged (mild / moderate / severe), other: _____

Cortex (outside layer): pink, tan, red, purple, other: _____

Medulla (inside layer): pink, tan, red, purple, other: _____

Left: normal, congested, hemorrhage, abscess, parasites, cysts, hydronephrosis (distended), mass, calculi, emboli, infarct, loss of renule differentiation, other: _____

Size: normal, small, enlarged (mild / moderate / severe), other: _____

Cortex (outside layer): pink, tan, red, purple, other: _____

Medulla (inside layer): pink, tan, red, purple, other: _____

Collect kidney in each Tissue Set, as well as two 4x4cm samples for toxicology, and two 1x1x1cm samples for microbiology:

Kidney (FM) A (FM) B

Sp # _____ A Kidney 4x4cm (tox) (LN) C Kidney 1x1cm (micro) (LN)

B Kidney 4x4cm (tox) (LN) D Kidney 1x1cm (micro) (LN)

URETERS: NSF / NE dilated, tumors, abscesses, stones/calculi, hydroureter (distended due to obstruction), other: _____

URINE: Attach a sterile 18 g x1.5" needle to a sterile syringe, insert the needle into bladder, and draw on syringe to collect up to 5 mL.

Amount: _____ ml ; bloody, golden, yellow, pale yellow, clear, cloudy, purulent, other: _____

Sp # _____ Urine (LN)

URINARY BLADDER: empty, full, dilated, thickened, masses, hemorrhagic, ulcerated, necrotic, other: _____

Bladder (FM) A (FM) B

MALE REPRODUCTIVE TRACT **NSF / NE / NA**

PREPUCE: NSF / NE

PENIS: NSF / NE discolored, pustules, mass, torsion, laceration, plaque, other: _____

TESTES

Left: NSF / NE immature, mature, shrunken, enlarged, mass, cyst, hernia, other: _____

Right: NSF / NE immature, mature, shrunken, enlarged, mass, cyst, hernia, other: _____

Collect paired samples and place one in each Tissue Set. Also collect and fix anything appearing abnormal.

Penis (FM) A (FM) B

Left Testis (FM) A (FM) B

Right Testis (FM) A (FM) B

FEMALE REPRODUCTIVE TRACT **NSF / NE / NA**

VULVA: NSF / NE other: _____

VAGINA: NSF / NE enlarged, hemorrhagic, purulent fluid (pus), mass, mucus, plaques, other: _____

UTERUS: NSF / NE enlarged, hemorrhagic, purulent fluid (pus), mass, mucus, plaques, other: _____

CERVIX: NSF / NE enlarged, hemorrhagic, purulent fluid (pus), mass, mucus, plaques, other: _____

OVARIES:

Left: NSF / NE enlarged, shrunken, mass, cyst, corpora lutea (present / absent), follicles (present / absent), other: _____

Right: NSF / NE enlarged, shrunken, mass, cyst, corpora lutea (present / absent), follicles (present / absent), other: _____

Female Reproductive Tract (FM) A (FM) B

For pregnant females, aborted fetuses, or perinatal pup deaths, examine and collect umbilicus, placenta and fetus:

UMBILICUS (describe): _____

Umbilicus (FM) A (FM) B Sp# _____ Umbilicus (LN)

PLACENTA: Collect four 5cm x 1cm **full thickness** strips (extending through to include both the fetal and maternal side) representative of normal and any abnormal portions of the placenta. Fix one sample in each Tissue set and freeze the other two samples.

Placenta (FM) A (FM) B Sp # _____ Placenta A (LN) B (LN)

FETUS: Perform a complete necropsy if possible and use separate Necropsy Report Form.

A fetus or premature pup "P0" is defined as <75cm straight length; the pelage, whiskers, nails, or oral cavity not fully developed.

Fetus necropsied: Y / N Necropsy # _____ (PIFSC assigns)
Straight length: _____ cm Axillary girth: _____ cm
Mass: _____ kg Sex: M / F

If the fetus appears fresh, take one Dacron virology swab from both the throat and rectum *before* beginning fetal necropsy.

Sp # _____ Throat Swab (LN)

Sp # _____ Rectal Swab (LN)

SPINAL CORD

NSF / NE / NA

After cutting off the head, the spinal cord will be visible within the spinal canal. Fix one sample in each tissue set and freeze one.

Spinal cord (FM) A (FM) B Sp # _____ Spinal cord (LN)

BRAIN

NSF / NE / NA

Clean away tissue on the skull where the hacksaw will be cutting. Before cutting the skull, examine it carefully, **photograph** any fractures or blunt injuries and collect any injured portions of the skull. Attempt to remove the brain intact and handle gently. **Collect the brain even if it has liquefied.**

Sp # _____ Skeletal (collect injured skulls only) (DR)

If liquefied: Collect liquefied brain in a whirlpak or cryovials and freeze.

Sp # _____ Brain A (LN)

If the brain is whole: there is a tough covering (tentorium cerebellum) separating the cerebrum from the cerebellum that can be cut with scissors or scalpel. Once removed, split the right and left halves of the brain using a new scalpel blade.

Fix a sample from cerebrum, cerebellum and brainstem in each Tissue Set. Freeze two pieces of cerebrum in whirlpaks. Be sure to collect representative samples of both normal and abnormal brain tissue in formalin and whirlpaks.

CEREBRUM: NSF / NE Congested, abscess, pus, hemorrhage, asymmetrical, edema, other: _____

CEREBELLUM: NSF / NE Congested, abscess, pus, hemorrhage, asymmetrical, edema, other: _____

Cerebrum (FM) A (FM) B Sp # _____ Brain A (LN) B (LN)

Cerebellum (FM) A (FM) B

Brainstem (FM) A (FM) B

DURA MATER and SKULL

NSF / NE / NA

Examine the inside of the skull (the side against the brain) for any evidence of fractures or hemorrhage (discrete regions of black/purple discoloration that may look like grape jelly). Thoroughly photograph any that are found. Collect any injured portions of the skull.

SKULL: discolored (describe: _____), pus, hemorrhage, congested, other: _____

The mandible (lower jaw) and all teeth within it should be collected and placed in a whirlpak for **unknown age animals only**.

Sp # _____ Skeletal (mandible) (DR)

Reminder:

24-48 hours after necropsy, pour off formalin from all formalin fixed tissues, rinse the tissues in fresh water, and store them in afresh 10% formalin solution until transporting tissues to Honolulu. Just before transport, all formalin should be poured off and transported in carboys. If necropsy is conducted within the 24-48 hour period, it is ok to transport the tissues in formalin, but be sure to pack them with enough absorbent material to soak up all formalin in case of leakage and clearly label any buckets containing formalin (for more details see "Packing and Shipping" section of Specimen Collection Protocol).

HAWAIIAN MONK SEAL *PARTIAL* NECROPSY REPORT FORM (for code 4 & 5 carcasses only)

If there is **any** doubt whether to use this form of the full form, **USE THE FULL FORM** and do as thorough a necropsy as possible.

Assign a necropsy number to ALL seals found dead in the season, whether or not a necropsy is actually performed.

SEAL ID/temp ID _____ **DEATH/NEC. #** _____ *(assign sequential #'s by calendar date for all seals)*
Date/time: necropsied _____ found dead _____ last seen alive _____
 Island/Atoll _____ Islet _____ Sector _____ Lat _____ Long _____
 Beach position _____ Carcass orientation (i.e., horizontal to water line) _____
 Size/sex _____ Age _____
 Persons performing/assisting with necropsy: _____
RECORDER: _____ **PHOTOGRAPHER:** _____
 Photos? Y / N File names: _____

HISTORY

Identifying body markings (take photos): _____

Last live observation(s): _____

Circumstances of death (found dead/euthanized/other-explain): _____

Tags

Record number and condition of any flipper tag(s), photograph, and collect all tags in labeled whirlpak.

R _____ L _____ Notes: _____

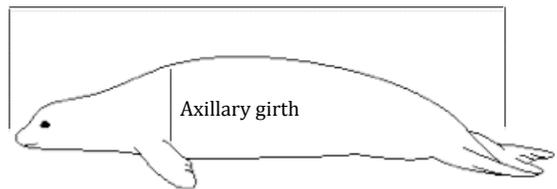
Scan the entire body for pit tags. PIT tag#(s):

R _____ L _____ Location found _____

Morphometrics

Axillary girth _____ (cm)
 Straight length _____ (cm, circle: DSL or VSL)
 Total body mass _____ (circle: kg/lb)
 Measurer _____

Straight Length (tip of nose to tip of tail)



Carcass Condition Code: 4 5

Code 4: Poor/advanced decomposition (carcass collapsed, skin slough
 liquified organs, blood thin and black, viscera friable difficult to dissect and easily torn, gut filled with gas)
 Code 5: mummified/skeletal remains (skin draped around bones, remaining tissues desiccated)

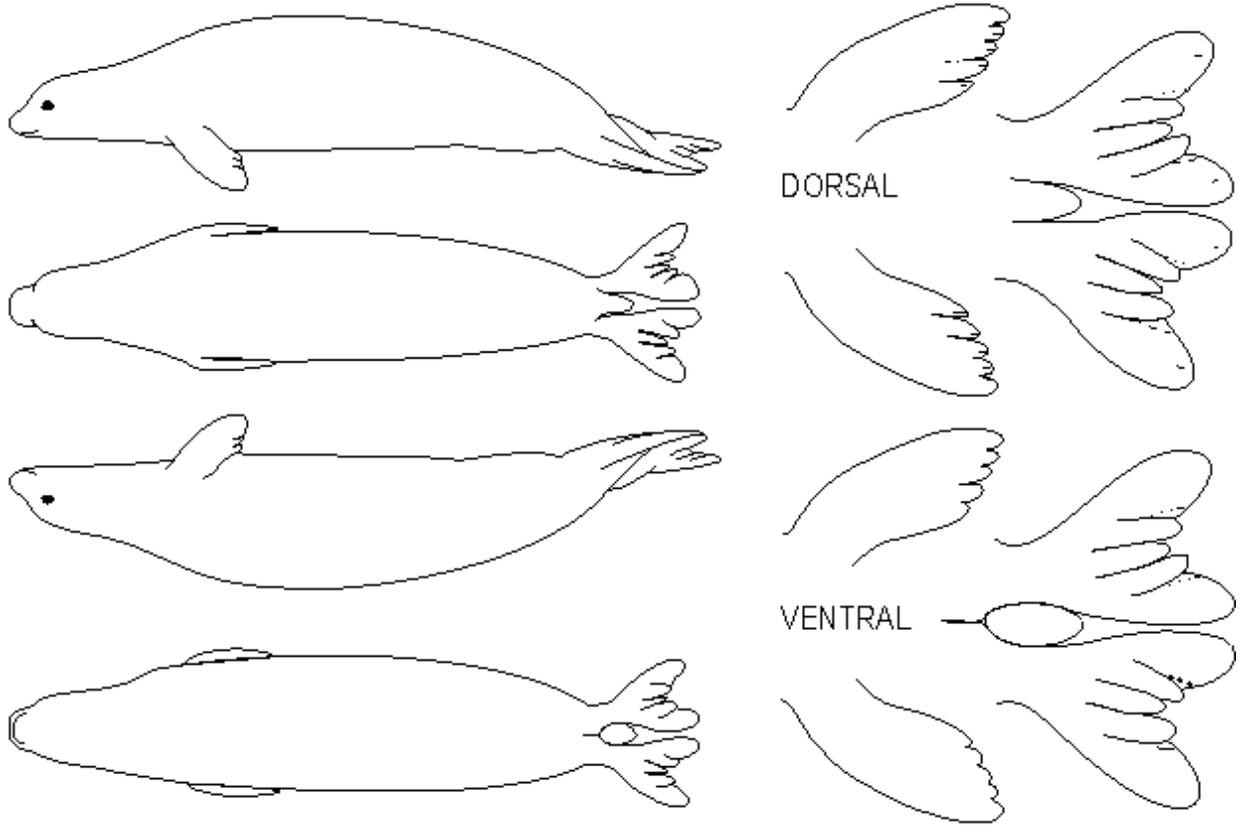
GROSS NECROPSY EXAMINATION

INSTRUCTIONS

- Describe all organs, tissues and lesions in the fields provided. If appropriate, circle either NSF, NE or NA at the top of each section. If the tissue examined is abnormal, describe the location, color, size and distribution within the tissue in the blanks provided.
- A sample of any visible endoparasites should be collected in alcohol per protocol below.
- If possible, remove head and place in cooler on ice while remainder of necropsy is completed, then go back and sample brain after the remainder of the necropsy is complete.
- Examine all outside surfaces and note abnormalities, discharge, etc. Describe/draw abnormalities below. Photograph with full-frame views of all 4 sides (dorsal/ventral/left lateral/right lateral) as well as close-ups of scars or other distinguishing characteristics, injuries, abscesses, line marks or other abnormalities. Include an index card in the image frame that notes the following: Seal ID, Date, Size, Sex, SF#, Necropsy #, Location (island/atoll).

NOTE: In cases of drowning, there are few clues, if any, so look closely for external indications of entanglement (bent/missing vibrissae, torn/missing nails, cuts in and around the nose, mouth, and gums). Also examine the tips of all extremities to look for line or net marks. Describe/draw and photograph any evidence of entanglement. Collect any gear found on an animal in a ziploc bag and label.

Draw all abnormalities and markings (include bruises, wounds, contusions, old scars, condition of and tears of skin, external parasites):



ABBREVIATIONS	
NSF: no significant finding	DB: DMSO/blue ice
NE: not examined	DR: dry

EXTERNAL PHYSICAL EXAMINATION (Circle all that apply):

GAS/ PUTREFACTION: (<i>Run your hands firmly along the body and feel for bubbles.</i>) NE / NA	NSF /
---	--------------

NUTRITIONAL STATE: Fat/overweight, Normal/average, Thin/poor, Starving/emaciated Notes: _____
--

DISCHARGE: NE / NA	NSF /
Location: eyes, nose, mouth, genitals, anus Color/Texture: green, white, clear, red, thick, runny Notes: _____	

EYES: NE / NA	NSF /
NSF, NE, missing, bulging, deflated, out of socket, foreign body present, penetrating wound Notes: _____	

MUCOUS MEMBRANES: NE / NA	NSF /
Pink, pale pink, red, yellow, white, purple, brown, other: _____	

ORAL CAVITY: NE / NA	NSF /
Ulcers, vomitus, blood, foreign body, other: _____	

TEETH: NE / NA	NSF /
Unerupted, just erupting, fully erupted, missing, broken, worn (describe): _____ LEFT (top/bottom): # incisors ___/___, # canines ___/___, # post-canines ___/___ RIGHT (top/bottom): # incisors ___/___, # canines ___/___, # post-canines ___/___	

VIBRISSAE: NE / NA	NSF /
Absent, torn, other: _____ Collect two vibrissae (with roots) and freeze in a whirlpak Sp# _____ Vibrissae <input type="checkbox"/> (LN)	

NAILS: NE / NA	NSF /
Absent, torn, bleeding, cracked, crushed, other: _____	

PERIPHERAL LYMPH NODES: NE / NA	NSF /
<i>Feel around the point of the shoulder (prescapular LN), and the angle of the jaw (mandibular LN)</i> Palpable, obvious, unnoticeable, other: _____	

HAIR COAT: NE / NA	NSF /
Hair missing, oil, molting, scruffy, parasites, foreign bodies, fishhooks, scavenging, abrasion, other: _____	

SKIN: **NSF /**
NE / NA

Cracking, bleeding, dry, moist, smooth, rough, wounds, masses, vesicles, ulcers, scars, bruising, abscesses, masses, abnormal coloration, other: _____

Collect two 1x1cm flipper tips in a 2mL cryovial pre-filled with 1.8 ml *DMSO* then freeze. **For the proper ratio of *DMSO* to tissue, please ensure that samples approximate the size of a pencil eraser.

Sp# _____ A TP (DMSO/LN)

Collect any skin lesions and freeze in a whirlpak.

Describe lesions:

Skin lesion(s) (LN)

MUSCLE **NSF / NE / NA**

Describe abnormalities: _____

Collect one 1x1x0.5cm piece of muscle for stable isotope analysis and freeze.

Sp# _____ A MU (LN)

OTHER NOTES ON EXTERNAL EXAMINATION:

INTERNAL EXAMINATION:

Thickness (measure on midline of chest between the front flippers): _____ mm

BLUBBER **NSF / NE / NA**

Thickness (measure on midline of chest between the front flippers): _____ mm

STOMACH **NSF / NE / NA**

Stomach contents: empty, dilated with gas, milk, mucus, fish (digested / partially digested / undigested), foreign body, other: _____

Collect stomach contents into whirlpaks or cryovials and chill on blue ice then freeze.

Stomach contents Sp# _____ (LN)

BRAIN **NSF / NE / NA**

Collect the brain even if it has liquefied. Before cutting the skull, follow directions below under the skull section. When opening the skull to collect the brain, be sure to collect the injured portions of the skull without damaging them. To prevent tissue from clogging the teeth on the saw, first clean away any tissue on the skull where the hacksaw blade will be cutting.

Describe: _____

If the brain has decomposed to the point that it has liquefied, collect in a whirlpak or cryovials and freeze.

Sp # _____ A Brain (BI)

B Brain (BI)

SKULL

NSF / NE / NA

Examine the skull carefully and **photograph** any fractures or blunt injuries. Collect any injured portions of the skull. Examine the inside of the skull (the side against the brain) for any evidence of fractures or hemorrhage (discrete regions of black/purple discoloration that may look like grape jelly). Thoroughly photograph any that are found. Collect any portions that appear injured.

SKULL: discolored (describe: _____), pus, hemorrhage, congested, other: _____

The mandible (lower jaw) and all teeth within it should be collected and placed in a whirlpak for **unknown age animals only**.

Sp # _____ Skeletal (mandible) (DR)

Sp # _____ Skeletal (collect injured skulls only) (DR)

If a Fetus is found – follow instructions below**Fetus (Describe):**

If fetus is <25 cm in length, split it in half from chin to pubis and place both halves in formalin. If fetus is >25 cm in length, **perform a complete necropsy and use the full Necropsy Report Form.**

If complete necropsy is performed, use full Necropsy Report Form. Handle tissues in a sterile manner. Take one swab from both the throat and rectum before beginning necropsy. **Priority samples (if available), regardless of condition code: Brain, lung, liver, stomach/stomach contents and placenta.**

If possible, collect several cc's of fluid from the stomach and freeze in liquid nitrogen. *Record this information on the full Necropsy Report Form.*

Ventral Length: _____ cm

Axillary Girth: _____ cm

Mass: _____ kg

Sex: M or F

Condition (Describe):

Fetus Necropsied: Y or N (if Y, see Necropsy # _____)

If Fetus is < 25 cm:

OR

If Fetus is > 25 cm:

Sp # _____ A Fetus (WC) (FM)

Sp # _____ Throat Swab (LN)

B Fetus (WC) (FM)

Sp # _____ Rectal Swab (LN)

Sp # _____ Stomach Fluid (LN)

MHI HAWAIIAN MONK SEAL NECROPSY PROTOCOL

SAFETY CONSIDERATIONS

Before performing a necropsy, read the following documents:

“Appendix II: Infectious Agents” (Aguirre, *et al.*, 1999) at the end of this document and/or in the tagging/handling section of your manual.

“Marine Mammal Zoonotic Bacteria” available in the “Visual Monk Seal” and online at:
<http://www.vetmed.ucdavis.edu/whc/mmz/bacteria.htm#Marine%20Mammal%20Zoonotic%20Bacteria%A0>

“Working with Marine Mammals and Your Health” available in the “Visual Monk Seal” and online at:
<http://www.vetmed.ucdavis.edu/whc/mmz/Occupational%20Safety.htm>

“Assessment of the Risk of Zoonotic Disease Transmission to Marine Mammal Workers and the Public: Survey of Occupational Risks” available in the “Visual Monk Seal” and online at:
http://www.sefsc.noaa.gov/PDFdocs/Marine_Mammal_Zoonoses_Final_Report.pdf

Preventing Disease Transmission

Avoid direct contact with dead seals to prevent transmission of infectious diseases that may be pathogenic to humans.

Persons performing the necropsy must:

9. Cover all surface wounds with a protective dressing before gearing up.
10. Wear protective gear, including latex or vinyl gloves, mask, disposable gowns, and foot covers. Change torn gloves **immediately**.
11. Seek medical attention immediately if you get any cuts, punctures or other injuries during the necropsy. Notify the attending physician of the source of the injury.
12. Disposable items such as scalpel blades, needles and biopsy punches **MUST** be disposed on in the sharps containers.
13. If possible, pull carcass up the beach to higher ground and bury it after necropsy to avoid attracting scavengers and minimize the potential for disease transmission.
14. Disinfect all instruments and contaminated equipment after the necropsy has been performed (see Post Necropsy section, below).
15. Once the necropsy has been performed and all gear has been cleaned and disinfected, wash thoroughly with soap. Disinfect reusable clothing with bleach solution (see tagging handling protocol) and dispose of all contaminated clothing, gloves, gowns, etc in a biohazardous waste bag.
16. **DO NOT STORE ANY SPECIMENS IN FREEZERS/REFRIGERATORS USED FOR HUMAN FOOD.**

GENERAL CONSIDERATIONS

A necropsy is a systematic examination of the whole body, organs, and tissues and is a basic tool for investigating disease and for monitoring the health of the Hawaiian monk seal population. **Whenever possible, necropsies should be performed by a trained veterinary pathologist** experienced in recognizing and interpreting lesions and abnormalities.

Necropsy How-To Guides:

For general guidance on the steps in performing a necropsy, please refer to the following resources, but follow the sample collection protocols provided in this document and the most recent version of the Necropsy Report Form.

3. "Field Manual for Phocid Necropsies (specifically *Monachus schauinslandi*)" (FMPN)
4. “Marine Mammal Necropsy: An Introductory Guide for Stranding Responders and Field Biologists” – available at: <https://darchive.mblwhoilibrary.org/handle/1912/1823>

Necropsies will have the most scientific value when they are carefully documented. Adherence to this protocol and the Necropsy Report Form will assist in the documentation and standardization of information, which may be valuable in determining morbidity and mortality factors within the population and as well as for individual seals.

Things to keep in mind:

1. Record all observations – when in doubt, just describe what you see.
2. The order of the Necropsy Report Form follows the sequence of general dissection and examination. If you are skilled and familiar with Hawaiian monk seal necropsies, you may find it easier to use the Necropsy Specimen Checklist, but **be sure to have someone record all observations, photos, measurements, and descriptions of organs on the Necropsy Report Form.**
3. Tissues and organs must be examined in a systematic manner. The precise method used for a necropsy is less important than establishing a routine in which each body system is examined fully.
4. **Once the carcass has been opened, take tissue specimens for virology, bacteriology and toxicology first, then sample for histopathology and parasitology.**
5. Samples of **normal and abnormal** tissue should be collected for laboratory analyses.

The ability to obtain reliable data from necropsies depends on the following:

1. Condition and location of the carcass
2. Adherence to detailed protocols
3. Number of seals necropsied throughout the year
4. Amount of time available to perform a thorough necropsy
5. Care in sample preservation and labeling of specimens
6. Care in shipping and storing specimens

Decomposed carcasses may be unsuitable for histopathology but can be useful for observing gross lesions. Collect brain samples regardless of the state of decomposition. Collect samples from all organs listed, even those that appear normal. In general, tissue specimens must be sufficiently thin (**less than 1 cm thick**) to allow proper fixing of 10 parts 10% buffered formalin: 1 part tissue. For some tissues (e.g. brain and lung), you may need to make parallel cuts (0.5 cm in thickness) in the tissues to allow preservation. After the tissues have been fixed in formalin 24-48 hours, pour off the formalin, rinse the tissues in fresh water, and add fresh formalin solution.

NECROPSY INSTRUCTIONS

Complete a Hawaiian Monk Seal Necropsy Report Form for **all** carcasses recovered. Use the full form if you perform an internal examination of the carcass, regardless of the condition code. The partial form can be used for necropsies where very minimal data is collected. Record "N/A" for any sections that are not applicable, and state what organs/tissues were not examined. At a minimum, describe each organ examined and sample as many organs as possible, prioritizing the following tissues: brain, lung, liver, kidney, blubber.

Photograph the exterior for ID (even if tagged), to document injuries or other unusual conditions, and to document body condition. Photograph the seal from all 4 sides (dorsal/ventral/left lateral/right lateral) and a close up of the hind flippers with tags. In addition, take close-ups and a wider view (to show perspective) of injuries and unusual conditions. If possible, include an index card in each frame that notes the following: Seal ID, Date, Size, Sex, and island and a ruler. Record photographs on the Necropsy Report Form.

External examination

Document any specific external lesions, abnormalities, or scar patterns. Examine, describe, and photograph any external lesions or injuries, the anogenital area, scars and other distinguishing characteristics.

Experience has shown that in cases where pinnipeds have drowned, there is often a complete absence of expected gross and histological findings. For this reason, it is imperative to look closely for external indications of entanglement. Findings may include: bent or missing vibrissae, torn or missing nails, and cuts in and around the nares, mouth, and gums. Closely examine the tips of all extremities to look for line or net cuts. Linear marks on the pelage are also of interest. Photograph any suspected abnormalities with close up/macro images, followed by images that demonstrate the location(s) on the body of each close up image.

Carcass condition codes

Evaluate carcass condition (state of decomposition). Carcass condition is influenced by many factors including disease, body temperature, and environmental temperature. **Rigor mortis** (stiffening of the body following death) may serve as an indicator of carcass evaluation. It can occur within hours in warm weather, but is extremely variable. *Rigor mortis* indicates that a carcass may be in good condition (Code 2).

Code 1: just died (e.g., euthanasia)

Code 2: fresh/carcass in good condition (rigor mortis, fresh smell, normal appearance, minimal drying of skin and mucous membranes, eyes clear, carcass not bloated, muscles and blubber firm, viscera intact and well-defined, guts with no gas). NOTE: Rigor mortis (stiffening of the body following death) may serve as an indicator of carcass evaluation. It can occur within hours in warm weather, but is extremely variable. Rigor mortis indicates that a carcass may be in good condition

Code 3: fair/decomposed (carcass and organs intact, bloating, skin sloughing, mild odor, eyes sunken, dried mucous membranes, friable viscera, blubber oily, muscles soft but still intact, gut dilated with gas)

Code 4: poor/advanced decomposition (carcass may be intact but collapsed, skin sloughing, strong odor, blubber soft with pockets of gas, liquified organs, blood thin and black, viscera friable difficult to dissect and easily torn, gut filled with gas)

Code 5: mummified/skeletal remains (skin draped around bones, remaining tissues desiccated)

Tags

If flipper tags are present, note their condition on the survey form (data type 'T') and tag condition forms. Collect and place them in a whirlpak bag labeled with animal ID, island/atoll, date, and survival factor number. Scan the entire body for PIT tags by holding the PIT tag reader as close to the body as possible. Even if PIT tags are not found, indicate on the survey sheet that a scan was completed and where on the body the scan was performed.

Morphometric measurements

Axillary girth – At the armpit, measure the circumference around the entire body in centimeters.

Standard length – Measure the straight line (not curved) length of the entire seal from the tip of the nose to the tip of the tail in centimeters. If a scale is available, weigh the body and report units. **Record measurements on both the TAGGING/HANDLING CARD and the Necropsy Report Form.**

Swab Collection

Use sterile Dacron swabs. Avoid touching swab tip to anything other than the tissue being swabbed. Immediately place swab in cryovial and break off the end of the plastic applicator against the side of the cryovial container (it should snap easily).

Internal Examination

TAKE INTERNAL PHOTOGRAPHS ONLY WHEN UNUSUAL CONDITIONS ARE NOTED OR

IF YOU ARE UNSURE IF IT IS UNUSUAL. If unusual conditions are noted, include a size reference (*e.g.*, ruler) and label with seal ID, survival factor number, date, and island. Take two photographs, one with the organ *in situ* (in its anatomical position/location) in the body and one with the organ removed from the body and placed on a solid white or light blue surface.

Record complete and thorough observations. Assume more is better when describing and recording information. The rule here is if in doubt, write it down. If unsure whether something is abnormal, state this and succinctly describe. Descriptions should be clear, concise, and without personal interpretation. Appropriate tissue preservation along with YOUR precise description of findings may allow the identification of causes of death in the population.

Identify the appropriate descriptors for each organ examined. The descriptions provided herein are NOT an exhaustive list of terms, but rather a list for your reference. **Describe surface, consistency, color, and cut surface of both normal tissues and abnormalities or lesions.**

Descriptors of Organs and Lesions

Surface: Smooth, rough, shiny, dull, thickened, wrinkled.

Consistency: Firm, soft, flabby, dry, wet, fluid-filled, sharp-edged, friable (easily pulverized or crumbled).

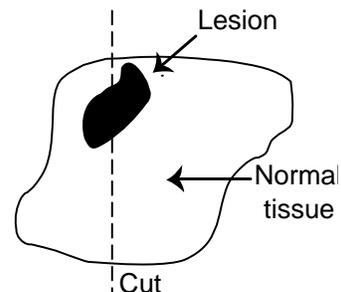
Color: Transparent, translucent, opaque; white, cream, green, yellow, brown, pink, red, nutmeg (normal pattern of liver), etc. Use simple colors, do not get complicated. Also comment how color is spread through tissue- homogeneous, speckled, streaked, blotchy, blanched, mottled (*i.e.*, pink with specks of red). Additional descriptors may include bright, pale, dark.

Cut surface: Slice organ several times appropriately and spread apart to look at internal surface. Be sure to describe color of the cut surface. Descriptors include swollen, bulging, shiny, dull, eroded, glistening, scaly,

Size:	pitted, oozing Record in metric system (mm, cm), measure length, width and depth or diameter of the lesion. Enlarged, (hypertrophied), small (atrophied), normal size.
Shape:	Square, rectangular, triangular, oval, round, cuboidal, spherical, discoid, rhomboid, tear-shaped, wedge-shaped, spindle-shaped, irregular, long, slender, indented, narrow, lace-like, tortuous, branching, speckled (miliary), flat, raised, depressed, shrunken, papillary, cauliflower-like.
Distribution:	Single discrete lesion (focal), multiple lesions in one location (multifocal), or multiple lesions scattered diffusely throughout the organ or body cavity (diffuse); locally extensive, random, even.
Location:	Surface, capsule, wall, dorsal or ventral, caudal or cranial, anterior or posterior, medial or lateral, proximal or distal, internal or external, full or partial thickness of a wall of an organ.
Fluid:	Clear, cloudy, turbid, thick, thin, bloody, mucoid, exudate, dark, tarry
Consistency:	Spongy, granular, gel-like, firm, soft, hard, rock-hard, dense, creamy, buttery, brittle, lumpy, velvety, warty, tenacious, gritty.
Cut surface:	Bulging, engorged, granular, nodular, pitted, oozing
Odor:	None, sweet, sour, rancid, ammonia-like, putrid, fruity, petroleum-like

Collecting necropsy tissues

Each complete necropsy should have two jars containing complete tissue sets of all tissues, and both having the same specimen number. One set should be sub# A and the other sub# B. Tissue set A should be the most complete set, (e.g. if you freeze one eye, tissue set A should have the formalin fixed eye). If there are any unusual lesions in any of the tissues sampled, be sure to include the margin between abnormal and normal tissue in both tissue sets A and B.



Collect samples of ALL LESIONS in formalin. Describe and sample areas that appear to stand out in marked contrast to the main body of tissue. Samples should include the margins between the normal and abnormal tissue and a description (i.e., sharp line versus vague and gradual, circumscribed, encapsulated). Make sure to check the boxes next to the appropriate specimens as collected on the necropsy report form.

Tissues for Toxicology (contaminants and biotoxins): Code 1, 2 ideal. Codes 3, 4, 5 useless.

Toxicological analyses may be performed for heavy metals, organochlorides, selenium, and dioxin. When sampling for toxicology, it is important to use standardized sampling procedures so that even when low levels of contaminants are present, differences may be attributed to biological processes and contaminant exposure and not to variation in the collection process.

1. Samples must be collected **less than 24 hours** from time of death. **Freeze at lowest temperature** available ASAP.
2. Tissue samples will be taken in duplicate (2 of each, except when noted).
3. Use a new, sterile blade for each organ sampled. Any stainless steel instruments used in contact with tissues should be cleaned with distilled water and rinsed with isopropyl alcohol before using if possible. **Each specimen should be rinsed with distilled water (if possible), wrapped in aluminum foil (with dull side touching specimen), placed in whirlpaks or ziplocks, then frozen.**
4. If carcass is fresh dead (code 1, 2), collect whole, heparinized blood. Use sterile syringe and needle to collect **uncoagulated** (not clotted) blood and place in GTT (green-top, heparinized vacutainer).
5. Avoid salt water, tobacco smoke, bug sprays, and other aerosolized foreign materials during collection.
6. **Tissues should be collected as rapidly as possible** after opening the body cavity to prevent contamination and deterioration.

Tissues for Microbiology: Code 1 ideal; Codes 2, 3 limited; Codes 4, 5 useless

Collect the following by special request only. Specimen collection for bacteriology and virology is determined primarily by the nature of gross pathologic lesions. Samples should be taken aseptically, from external surfaces, body cavities and internal organs as soon as they are exposed. Place swabs in respective transport media and refrigerate at 4 C or place on blue ice immediately and freeze upon arrival to laboratory or field camp. If cryovials are available, ultrafreeze the swabs with tissue samples in liquid nitrogen. Samples for microbiology are worth the time and effort only when tissues are in suitable condition. With an "aborted fetus", perinatal death, or newborn, collect specimens according to "Fetus" section of Necropsy Form for freezing and later microbial analysis.

Post Necropsy

9. Review the completed Necropsy Report Form, making sure that all boxes have been checked off on the form for all samples collected.
10. Necropsy Report Forms, photos, "List of Specimens Collected", and any other pertinent data should be returned to the NMFS PIFSC Honolulu Laboratory.
11. Refer to the section "Preventing Disease Transmission" #4-8 on page one for post-necropsy clean-up tips. Clean necropsy tools (you may also need to spray them with WD-40 or LPS) and restock necropsy kit so that it is ready for the next necropsy.
12. Change the formalin for all formalin fixed tissues as noted above.
13. Make sure that the tagging/handling card, scar card, and tag condition drawing form are complete. Necropsy Report Forms, scar cards, tagging/handling cards, survival factor forms, and photos should be returned to the NMFS Honolulu Laboratory.
14. Record specimens collected on the Specimen Collection Summary and assign specimen numbers as outlined in the **Specimen Collection Protocol**.
17. Clean necropsy tools. Before disinfecting, remove all organic matter from instruments by washing them thoroughly with warm (if possible) soapy water. If instruments are not cleaned properly before disinfecting, the remaining organic matter may shield organisms from destruction, and may inactivate the disinfectant. Be sure to wear proper protective gear (gloves, masks, etc.) when washing instruments. To minimize aerosolization, keep instruments below the water line when washing. Disinfect instruments with 70% alcohol or a 1:10 chlorine bleach solution for at least **10 minutes**. However, bleach corrodes stainless steel, and may pit the instruments. Regardless of disinfectant used, be sure to thoroughly rinse instruments with fresh water after disinfecting. Air dry all instruments thoroughly before putting them away. You may also need to spray them with WD-40 (or LPS)
18. Restock necropsy kit so that it is ready for the next necropsy.

HAWAIIAN MONK SEAL NECROPSY REPORT FORM (MHI)

SEAL ID/temp ID _____ NEC. DATE/ TIME _____ NEC. # _____ (PIFSC assigns)
Date/time found dead _____ Date/time last seen alive _____
Island _____ Location _____ Lat _____ Long _____
Size/sex _____ Age (if fetus, see pg. 19) _____
DVM/ PROSECTOR(S): _____
RECORDER: _____ **PHOTOGRAPHER:** _____
Photos? Y / N File names: _____
X-rays? Y / N Findings: _____

HISTORY

Identifying body markings (take photos): _____

Last live observation(s): _____

Circumstances of death (found dead/euthanized/other-explain): _____

Tags

Record number and condition of any flipper tag(s), photograph, and collect all tags in labeled whirlpak.

R _____ L _____ Notes: _____

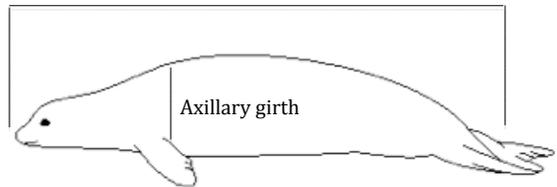
Scan the entire body for pit tags. PIT tag#(s):

R _____ L _____ Location found _____

Morphometrics

Axillary girth _____ (cm)
Straight length _____ (cm)
Total body mass _____ (circle: kg/lb)

Straight Length (tip of nose to tip of tail)



Carcass Condition Code: 1 2 3 4 5

- Code 1*: Just died (eg., euthanasia)
- Code 2*: Fresh, good condition (rigor mortis, fresh smell, normal appearance, minimal drying of skin/mucous membranes, eyes clear, carcass not bloated, muscles and blubber firm, viscera intact and well-defined, guts with no gas).
- Code 3: Fair/decomposed (carcass and organs intact, bloating, skin sloughing, mild odor, eyes sunken, dried mucous membranes, friable viscera, blubber oily, muscles soft but still intact, gut dilated with gas)
- Code 4: Poor/advanced decomposition (carcass collapsed, skin sloughing, strong odor, blubber soft w/ pockets of gas, liquified organs, blood thin and black, viscera friable difficult to dissect and easily torn, gut filled with gas)
- Code 5: mummified/skeletal remains (skin draped around bones, remaining tissues desiccated)

***For Code 1 and 2 carcasses:** Follow instructions below to collect blubber, liver and kidney for NIST. It is imperative that you adhere to the NIST Sampling Protocol and AVOID TOUCHING THESE TISSUES with regular gloves until NIST samples are collected.
If not sampled for NIST, explain: _____

GROSS NECROPSY EXAMINATION INSTRUCTIONS

1. Describe all organs, tissues and lesions in the fields provided. For descriptive terms, refer to Necropsy Protocol page 2. If appropriate, circle either NSF, NE or NA at the top of each section. If the tissue examined is abnormal, describe the location, color, size and distribution within the tissue in the blanks provided.
2. Formalin fixed tissues should be no bigger than 2x2x1cm.
3. Collect 2 equal Tissue Sets (set "A" and "B") from all organs, to be fixed in formalin.

Tissue Set A = Sp # _____ A Tissue Set B = Sp # _____ B

- 4. All visible endoparasites should be collected and chilled on blue ice.
- 5. If possible, remove head and place in cooler on ice while remainder of necropsy is completed.

ABBREVIATIONS		
NSF: no significant finding	BI: blue ice	AA=95% ethyl alcohol
NE: not examined	FM:10% formalin	AL=70-75% alcohol

EXTERNAL PHYSICAL EXAMINATION (Circle all that apply):

GAS/ PUTREFACTION: (Run your hands firmly along the body and feel for bubbles.) NSF / NE / NA

NUTRITIONAL STATE:
Fat/overweight, Normal/average, Thin/poor, Starving/emaciated
Notes: _____

DISCHARGE: NSF / NE / NA
Location: eyes, nose, mouth, genitals, anus Color/Texture: green, white, clear, red, thick, runny
Notes: _____

SWAB COLLECTION: NSF / NE / NA
Use sterile Dacron swabs. Obtain 2 swabs each from: eyes (ES), nasal cavity (NS), oral cavity (OR), genital opening (GS), rectum (RS). Both swabs from the same orifice can be placed into the same cryovial, unless the eyes are abnormal. If one or both eyes are abnormal, store the swabs separately and indicate below which vial contains the abnormal eye.

Sp # _____ Eye swab (BI) Sp # _____ Genital swab (BI) Sp # _____ Oral swab (BI)
Sp # _____ Nasal swab (BI) Sp # _____ Rectal swab (BI)

MUCOUS MEMBRANES: NSF / NE / NA
Pink, pale pink, red, yellow, white, purple, brown, other: _____

ORAL CAVITY: NSF / NE / NA
Ulcers, vomitus, blood, foreign body, other: _____

TEETH: NSF / NE / NA
Unerupted, just erupting, fully erupted, missing, broken, worn (describe): _____
LEFT (top/bottom): # incisors ___/___, # canines ___/___, # post-canines ___/___
RIGHT (top/bottom): # incisors ___/___, # canines ___/___, # post-canines ___/___

VIBRISSAE: NSF / NE / NA
Absent, torn, other: _____
Collect two vibrissae (with roots) and freeze in a whirlpak Sp# _____ Vibrissae (BI)

EYES: NSF / NE / NA
NSF, NE, missing, bulging, deflated, out of socket, foreign body present, penetrating wound
Notes: _____

Aqueous humor: collect using sterile needle and 3cc syringe. If both eyes are normal, aqueous can be combined in one cryovial. If one or both eyes are abnormal, use a clean needle and syringe to collect each sample and place in separate cryovials. Indicate which eye is abnormal above.

Sp # _____ Aqueous humor (BI)

Eyes: Collect both eyes. Fix one eye in Tissue Set A and freeze the other eye in a whirlpak. If an eye is abnormal, it should be fixed rather than frozen. If both eyes are abnormal, fix one in each Tissue Set. To fix an eye, make a 2-3 cm cut in the globe along the interface of the sclera (white part) and the clear cornea at the front of the eye before placing in formalin.

Eye (FM) A **AND** Sp # _____ Eye (BI) **OR** Eye (FM) B

NAILS: _____ **NSF / NE / NA**

Absent, torn, bleeding, cracked, crushed, other: _____

PERIPHERAL LYMPH NODES: _____ **NSF / NE / NA**

Palpate around the point of the shoulder (prescapular LN), and the angle of the jaw (mandibular LN)

Palpable, obvious, unnoticeable, other: _____

HAIR COAT: _____ **NSF / NE / NA**

Fur missing, oil, molting, scruffy, parasites, foreign bodies, fishhooks, scavenging, abrasion, other:

SKIN: _____ **NSF / NE / NA**

Cracking, bleeding, dry, moist, smooth, rough, wounds, masses, vesicles, ulcers, scars, bruising, abscesses, masses, abnormal coloration, other: _____

Collect two 1x1cm flipper tips in a 2mL cryovial pre-filled with 1.8 ml DMSO. For the proper ratio of DMSO to tissue, please ensure that samples approximate the size of a pencil eraser.

Sp# _____ A TP (DMSO)

Collect 2 samples from any masses, ulcers, vesicles or other external abnormalities. Fix one sample in Tissue Set A and freeze the other in a whirlpak. Try to include the junction of normal and abnormal tissue in the sample. Describe lesions sampled: _____

Skin lesion(s) (FM) Sp# _____ Skin lesion(s) (BI)

EXTERNAL GENITALIA & ANUS: _____ **NSF / NE / NA**

Swollen, protruding/prolapsed, diarrhea, ulcerations, masses, plaques, other: _____

Collect and fix anything appearing abnormal. Abnormal genital tissue (FM) A (FM) B

MAMMARY GLANDS: _____ **NSF / NE / NA**

Lactating, swelling, discharge, parasites, other: _____

If lactating, collect as much milk as possible and freeze. Sp # _____ Milk (BI)

INTERNAL EXAMINATION:

BLOOD: _____

For code 1 and code 2 animals ONLY, collect whole blood from extradural vein at least one (up to four) SST tubes. If unsuccessful, return to this step when you get to the heart. In the heart, look for a pale, tan, gelatinous “chicken fat clot” and separate it into RTTs. Centrifuge, aliquot serum and freeze as soon as possible.

Sp # _____ Whole blood or serum (CODE 1 and 2 animals only)

For code 1 carcasses ONLY, collect 2.5mL whole blood from extradural vein into a PAX gene tube. Gently rock tube to mix blood and additive. After blood collection, store tube UPRIGHT at ROOM TEMPERATURE for a minimum of 2 hours (longer is ok, just no more than 72 hours). After the 2 hour incubation, transfer sample to cryovial(s) using disposable pipette and place in -20C freezer for 24 hours. After 24 hours in -20C freezer, samples can be transferred to -80C freezer if desired. If no freezer is immediately available, tubes may be stored in a refrigerator for up to 5 days. Avoid placing samples directly from room temperature storage into the -80 freezer, as this will likely ruin the sample. NOTE: Unused PAX gene tubes should be stored at room temperature and not exposed to excessive heat or cold before use.

Sp # _____ Whole blood PAX gene tube (CODE 1 animals only)

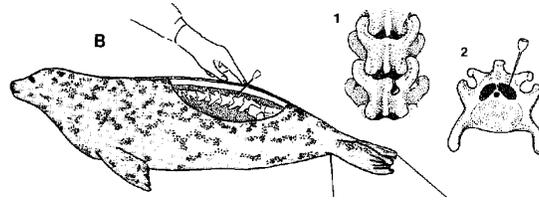
Extradural vein blood collection:

Palpate the vertebral column and pelvis and move your fingers cranially 2 or more vertebral spaces, feeling for a “divot” lateral to the spinous processes of the vertebrae. Attach a needle to a 3cc syringe.

Needle choice:

- Pups/weaners: use a 20g or 21g x 1 1/2” needle.
- Adults: use a 3.5” spinal needle. Before insertion, remove the stylet, holding needle from hub only.

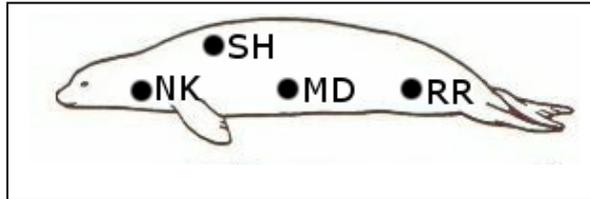
The angle of the needle may vary from a 45 - 90 degree angle to the dorsal surface of the animal. As the needle is inserted, feel it moving through skin, blubber, and muscle until you feel it pop through the membrane of the extradural sinus. Draw back on syringe to collect blood. Put a fresh needle (a small one is fine) on the syringe and push needle through top of PAX gene tube. The vacuum in the syringe should draw the blood into the tube.



BLUBBER

NSF / NE / NA

Thickness (measure at sternum or chest between the front flippers): _____ mm



NIST BLUBBER SAMPLING: For **code 1 and 2** animals only, using NIST provided materials (tyvek lab coat, **vinyl gloves**, **Teflon bags**, zip ties and data sheets) and stainless steel instruments, collect 300-350 g of blubber. Place in Teflon bag and seal with zip ties, label, and place on ice until it can be processed.

Sp# _____ NIST Blubber sample 300-350 g in Teflon bag (BI)

Code 1 and 2 animals only:

Using a twisting motion, insert a sterile 6 mm biopsy punch through the skin at the lateral aspect of the seal’s pelvic girdle, approximately 5-15 cm cranial to the wing of the ileum on either side of the body (RR, below). **Collect 8 blubber samples** through the **full depth** of the blubber layer, about 2-5 cm, until you reach the muscle layer. Use thumb forceps and/or scissors to retrieve the samples, without damaging or contaminating the blubber tissue. **One punch can be used to collect all 8 samples.** Take great care to prevent contamination of the samples by contact with gloves or other items. **The 4 best full thickness biopsies should go into the 5.0 ml cryovial, and the other 4 should go into the Teflon vial.**

Sp# _____ BB/FA from RR x4 (blubber biopsy for fatty acid in cryovial) (BI)

Sp# _____ BB/TX from RR x4 (blubber biopsy for toxicology in small Teflon vial) (BI)

All animals:

Collect two 1x1x1cm samples of skin and blubber from the RR location and fix one in each Tissue Set.

Skin/Blubber (FM) A (FM) B

Collect one **full thickness**, 2 x 2 cm blubber sample from each of the following locations: neck (NK), shoulder (SH) and midsection (MD) locations. Place each sample in separate whirlpak (do not wrap in foil), and label each bag with the body site from which it was collected.

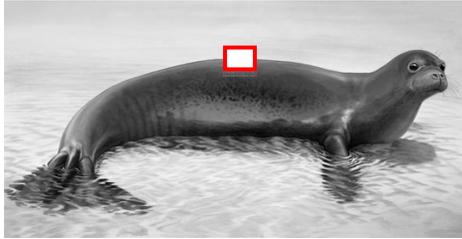
Sp# _____ BL/NK one 2 x 2 cm from NK (fatty acids) (BI)

Sp# _____ BL/MD one 2 x 2 cm from MD (fatty acids) (BI)

Sp# _____ BL/SH one 2 x 2 cm from SH (fatty acids) (BI)

MUSCLE **NSF / NE / NA**

Describe abnormalities: _____
 Collect the following samples from the middle of the the *longissimus dorsi*, the large long muscle that runs down either side of the spine:



One 2x2x2 inch muscle sample for myoglobin analysis. **Double bag** the sample in whirlpaks and freeze.

Sp# _____ A MU (BI)

One 1x1x0.5cm piece for stable isotope analysis and freeze.

Sp# _____ B MU (BI)

Two 1x1x1cm pieces in formalin and place one in each Tissue Set.

Muscle (FM) A (FM) B

CEREBRAL SPINAL FLUID (CSF) **NSF / NE / NA**

Cut through the skin, blubber, nuchal ligament, and neck muscles to the dorsal aspect of the atlantoccipital skull joint. Maintaining sterile technique, slowly insert needle w/ syringe approximately 5-7 mm into the foramen magnum. Collect up to 5ml and freeze.

Sp # _____ CSF (BI) sterile/ non-hemolyzed sample

Sp # _____ CSF (BI) contaminated/ hemolyzed sample

ORO/NASOPHARYNX **NSF / NE / NA**

NASOPHARYNX: Look for nasal mites. If observed, collect in alcohol. Sp # _____ nasal mites (AL)

Fluid (Y / N) volume: _____ ml. Describe fluid: _____

TONSILS: enlarged, red, purple, pus, other: _____

Ulcers on tongue, ulcers on gums, ulcers on hard palate, foreign body, vomitus (volume: _____ ml, appearance: _____)

THORACIC CAVITY

Open the chest and look for free fluid in the thoracic cavity (around the lungs, pooled at the diaphragm), collect 1-3ml if indicated (veterinary discretion) and describe below. Examine external surfaces of lungs and heart *in situ* and note abnormalities in appropriate section.

Fluid present (Y / N), volume: _____ ml, appearance: purulent, serous, fibrinous, yellow, white, green, blood-tinged, frank blood, adhesions, plaques, other: _____

Sp # _____ chest fluid (BI)

SALIVARY GLANDS: NSF / NE abnormalities: _____

THYROID: NSF / NE abnormalities: _____

LARYNX: NSF / NE abnormalities: _____

ESOPHAGUS: NSF / NE dilated, constricted, perforated, ulcerated, hemorrhagic, foreign body, fluid (volume: _____ ml, appearance: _____), other: _____

TRACHEA: NSF / NE perforated, lacerated, foam (mild / moderate / heavy), fluid (mucoïd / purulent / white / yellow / green / blood), volume: _____ ml. Mucosa: congested, hemorrhagic, ulcerated Notes: _____

BRONCHI: NSF / NE perforated, lacerated, foam (mild / moderate / heavy), fluid (mucoïd / purulent / white / yellow / green / blood), volume: _____ ml. Mucosa: congested, hemorrhagic, ulcerated Notes: _____

PARASITES: nematodes, mites, other: _____ location: _____ Severity: <10, 10-20, 20-50, >50

THYMUS: NSF / NE atrophy, prominent, enlarged, other: _____

Note: the thymus shrinks with age, and is not likely be found on adult animals.

Submandibular LN	<input type="checkbox"/> (FM) A	<input type="checkbox"/> (FM) B	Thymus	<input type="checkbox"/> (FM) A	<input type="checkbox"/> (FM) B
Tonsil	<input type="checkbox"/> (FM) A	<input type="checkbox"/> (FM) B	Esophagus	<input type="checkbox"/> (FM) A	<input type="checkbox"/> (FM) B
Tongue	<input type="checkbox"/> (FM) A	<input type="checkbox"/> (FM) B	Trachea	<input type="checkbox"/> (FM) A	<input type="checkbox"/> (FM) B
Thyroid	<input type="checkbox"/> (FM) A	<input type="checkbox"/> (FM) B	Bronchus	<input type="checkbox"/> (FM) A	<input type="checkbox"/> (FM) B

BRACHIAL PLEXUS **NSF / NE / NA**

If possible, collect the adjacent axillary LN with the brachial plexus and associated vessels and place in formalin.

Notes: _____

Brachial plexus (FM) A (FM) B

CRANIAL and THORACIC LYMPH NODES **NSF / NE / NA**

PULMONARY LYMPH NODES: Collect one sample in formalin and and freeze one for microbiology. Look around the base of the heart (they are located at the largest end of the blood vessels going to the lungs).

Pulmonary LN (FM) A (FM) B Sp # _____ PN (BI)

MEDIASTINAL LYMPH NODES: Collect one sample in formalin and freeze one for microbiology. Look around the heart and between the lungs. It is very difficult to determine the specific name of the node but all we are after are any abnormal lymph nodes and a few normal from the chest cavity

Mediastinal LN (FM) A (FM) B Sp # _____ mediastinal lymph node (BI)

OTHER LYMPH NODES: Examine as many additional cranial lymph nodes as possible: mandibular, axillary, prescapular, sternal. Describe any abnormalities below. Suggested descriptors: hemorrhagic, gelatinous, serous fluid, soft, hard, enlarged (mild/mod/severe).

Collect 1x1x1cm samples from abnormal nodes and preferably 2-5 normal nodes, label with a laundry tag and fix one sample from each node in each Tissue Set. Be sure to include abnormal and normal tissue in the fixed samples. For code 2+ carcasses, sample the freshest lymph nodes, label and place one sample from each node in each Tissue Set.

List lymph nodes collected in formalin: _____ (FM) A (FM)

HEART

PERICARDIUM: NSF / NE thickened, plaques on surface, contains fluid (volume: _____ ml, describe: _____)

Collect pericardial fluid if abnormal (veterinary discretion): Sp # _____ HF pericardial fluid (BI)

PULMONARY ARTERIES AND AORTA: NSF / NE thrombi, plaques, rupture, other: _____

VALVES*: NSF / NE diffusely thickened, nodular thickening, vegetative/proliferative lesion (valve(s): _____)

**If valves appear abnormal, place a sample in each Tissue Set.*

LEFT / RIGHT VENTRICLES: NSF / NE thickened, dilated (location _____ thickness: _____ mm)

MYOCARDIUM (heart muscle): NSF / NE pale, tumors, abscess, white foci (location: _____)

ATRIA AND AURICLES: NSF / NE thickened, dilated, pale, tumor, abscess, white foci

Parasites type: _____ severity: <10, 10-20, 20-50, >50

Notes: _____

Collect the following and fix one of each in each Tissue Set:

Section through L. ventricle - IVS - R. ventricle (FM) A (FM) B

Aorta (FM) A (FM) B

Pulmonary arteries (FM) A (FM) B

Section through R. atrium – AV valve (FM) A (FM) B

Section through L. atrium – AV valve (FM) A (FM) B

Next, collect 3 samples of heart tissue, wrap in foil, and freeze. REVISE FOR APPROPRIATE TOXICOLOGY NEEDS

Sp # _____ A Heart (BI)

B Heart (BI)

C Heart (BI)

LUNGS

NSF / NE / NA

Describe: pink, red, purple, mottled, congested, consolidated, abscesses, granulomas, emphysema, masses, interstitial edema

Specify location, distribution, severity: _____

Parasites: none detected 1 2+ 3+ 4+ Describe color, size: _____

Collect two 2x2x1cm pieces of lung and fix one in each Tissue Set. Collect additional samples if abnormalities are observed. Be sure to include both normal and abnormal tissue and describe abnormalities.

Lung (FM) A (FM) B

Collect 3 pieces of lung, 100 g each, wrap in foil, and chill on blue ice. REVISE FOR APPROPRIATE TOXICOLOGY NEEDS

Sp # _____ A Lung (BI)

B Lung (BI)

C Lung (BI)

ABDOMINAL CAVITY **NSF / NE / NA**

Open the abdomen and look for free fluid, collect 1-3ml if indicated (veterinary discretion) and describe below. Examine external surfaces of abdominal organs *in situ* and note abnormalities (adhesions, intussusceptions, etc.) in appropriate section.

Fluid present (Y / N), volume: _____ ml, appearance: purulent, serous, fibrinous, yellow, white, green, blood-tinged, frank blood, adhesions, plaques, other: _____

Sp # _____ abdominal fluid (BI)

DIAPHRAGM **NSF / NE / NA**

Collect two 2x2 cm samples of the diaphragm and fix one in each Tissue Set.

Diaphragm (FM) A (FM) B

LIVER **NSF / NE / NA**

NIST SAMPLING: For **codes 1 and 2** animals only, using NIST provided materials (tyvek lab coat, vinyl gloves, Teflon bags, zip ties and data sheets) and using stainless steel instruments, collect **300-350 g** of liver. Place in Teflon bag and seal with zip ties, label, and place on ice until it can be processed.

Sp# _____ NIST liver sample 300-350 g in Teflon bag (BI)

Describe: enlarged, small, tan, brown, black, yellow, orange, mottled, abscesses, granuomas, masses, cysts, hemorrhage, parasites, other: _____

Collect at least two 2x2x1 cm of liver and fix one in each Tissue Set. Be sure to also include both normal and abnormal liver in each Tissue Set.

Liver (FM) A (FM) B

Collect and freeze: three 100g liver samples, two 1x1x1cm samples and one 2x2x1cm sample and freeze.

Sp # _____	A liver 100 g (tox)	<input type="checkbox"/> (BI)	D liver 1x1x1 (micro)	<input type="checkbox"/> (BI)
	B liver 100 g (tox)	<input type="checkbox"/> (BI)	E liver 1x1x1 (micro)	<input type="checkbox"/> (BI)
	C liver 100 g (biotox)	<input type="checkbox"/> (BI)	F liver 2x2x1 (stable isotopes)	<input type="checkbox"/> (BI)

Collect the hepatic lymph nodes for microbiology, chill on blue ice. Look around the base of the large blood vessel coming from the aorta nearest the liver.

Sp # _____ hepatic lymph nodes (BI)

GALL BLADDER **NSF / NE / NA**

Describe: full, empty, thickened wall, flukes (severity: <10, 10-20, 20-50, >50), other: _____

Bile: thick/chunky, thin/runny, black, dark green, light green, yellow, orange, stones present

If present, collect 1-3ml of bile (code 1-2 carcasses only) and place in a cryovial and whirlpak.

Sp # _____ Bile (BI)

Collect two 2x2x1 cm sections of gall bladder and fix one in each Tissue Set.

Gall bladder (FM) A (FM) B

PANCREAS **NSF / NE / NA**

Loss of lobulation, swollen, hemorrhage, abscesses, other: _____

Collect two 2x2x1 cm pices of pancreas and fix one in each Tissue Set.

Pancreas (FM) A (FM) B

SPLEEN **NSF / NE / NA**

Masses, enlarged (mild / moderate / severe), constricted, congested, abscesses, scars, pale, purple, brown, red, other: _____

Collect tissues for 2 sets for histopathology and for 2 for microbiology (1x1x1 cm):

Spleen (FM) A (FM)

Sp# _____ A spleen (BI)

B spleen (BI)

STOMACH **NSF / NE / NA**

Erosions, ulcers, perforated ulcers, loss of rugal folds, swollen rugal folds, other: _____

Mucosa: white, pale pink, red, purple, other: _____

Parasites (ascaris): <10 10-20 20-50 >50 Describe (size, color): _____

Collect representative sample of parasites and freeze.

Parasites Sp# _____ (BI)

Stomach contents: empty, dilated with gas, mucus, fish (digested / partially digested / undigested), foreign body, other: _____

Collect stomach contents into whirlpaks or cryovials and chill on blue ice.

Stomach contents Sp# _____ (BI)

Collect two 2x2x1 cm sections of stomach tissue and fix one in each Tissue Set. Be sure to also include any abnormal tissue.

Stomach (FM) A (FM) B

INTESTINES **NSF / NE / NA**

Open up ~4-6" of each section of the gastrointestinal tract, and look for abnormalities in color or thickness. Be sure to include any abnormal tissue as well as normal tissue in the formalin fixed samples. For intestinal samples, take a complete transverse "ring" of tissue ~1-2 cm in width, trying not to touch or disrupt the inside of the ring.

Duodenum (FM) A (FM) B

Jejunum (FM) A (FM) B Cecum (FM) A (FM) B Colon (FM) A

(FM) B

Also collect samples (2 cubic inches) of small intestine for microbiology, chill on blue ice:

Small intestine Sp# _____ A (BI) B (BI) C (BI)

MESENTERIC LYMPH NODES:

Collect anywhere along the mesentery, preferably both at the cranial and caudal portions of the abdomen/intestinal tract. Collect 2 sets for tissue set A and B, and freeze several whole for microbiology. Chill on blue ice.

Mesenteric Lymph Node (FM) A (FM) B Sp # _____ ML (BI)

FECAL SAMPLES: Cut colon near anus and squeeze contents from distal intestines directly into container.

FE (feces-Intestinal Contents) Sp# _____

E. Collect sub-sample in pre-filled DMSO vial, fill to the 5 ml line on the vial Feces A (DMSO)

F. Collect 1-3 g in a whirlpak for ciguatera analysis (FE/CX) Feces B (whirlpak)

G. Freeze an additional 10-30 g in whirlpaks or cryovials for hormonal studies (FE/HR) Feces C (whirlpak/cryo)

H. Freeze 10 g of feces (or meconium if newborn pup) into whirlpaks or cryovials Feces D (whirlpak/cryo)

ADRENAL GLAND

Right: NSF / NE enlarged, shrunken, hemorrhagic, abscessed, dark, pale, other: _____

Left: NSF / NE enlarged, shrunken, hemorrhagic, abscessed, dark, pale, other: _____

Region (cortex vs medulla) and distribution of lesions: _____

Adrenal gland (FM) A (FM) B Sp # _____ Adrenal (BI)

URINARY TRACT **NSF / NE / NA**

NIST SAMPLING: For **codes 1 and 2** animals only, using NIST provided materials (tyvek lab coat, vinyl gloves, Teflon bags, zip ties and data sheets) and using stainless steel instruments, collect 300-350 g of kidney. Place in Teflon bag and seal with zip ties, label, and place on ice until it can be processed.

Sp# _____ NIST Kidney sample 300-350 g in Teflon bag (BI)

Right: normal, congested, hemorrhage, abscess, parasites, cysts, hydronephrosis, mass, calculi, emboli, infarct, loss of renule differentiation, other: _____

Size: normal, small, enlarged (mild / moderate / severe), other: _____

Cortex (outside layer): pink, tan, red, purple, other: _____

Medulla (inside layer): pink, tan, red, purple, other: _____

Left: normal, congested, hemorrhage, abscess, parasites, cysts, hydronephrosis, mass, calculi, emboli, infarct, loss of renule

differentiation, other: _____
Size: normal, small, enlarged (mild / moderate / severe), other: _____
Cortex (outside layer): pink, tan, red, purple, other: _____
Medulla (inside layer): pink, tan, red, purple, other: _____

Collect kidney in each Tissue Set, as well as two 100g samples for toxicology, and two 1x1x1cm samples for microbiology: Kidney

(FM) A (FM) B

Sp # _____ A Kidney 100g (tox) (BI) C Kidney (micro) (BI)

B Kidney 100g (tox) (BI) D Kidney (micro) (BI)

URINE: Attach a sterile 18 g x 1.5" needle to a sterile syringe, insert the needle into bladder, and draw on syringe to collect up to 12 ml.
Amount: _____ ml; bloody, golden, yellow, pale yellow, clear, cloudy, purulent, other: _____

Sp # _____ Urine (BI)

URINARY BLADDER: empty, full, dilated, thickened, masses, hemorrhagic, ulcerated, necrotic, other: _____

Bladder (FM) A (FM) B

URETERS: NSF / NE dilated, tumors, abscesses, stones/calculi, hydroureter (distended due to obstruction), other: _____

MALE REPRODUCTIVE TRACT

NSF / NE / NA

PREPUCE: NSF / NE

PENIS: NSF / NE discolored, pustules, mass, torsion, laceration, plaque, other: _____

TESTES

Left: NSF / NE immature, mature, shrunken, enlarged, mass, cyst, hernia, other: _____

Right: NSF / NE immature, mature, shrunken, enlarged, mass, cyst, hernia, other: _____

Collect a 2x2x1cm section of each tissue and place one in each Tissue Set. Also collect and fix anything appearing abnormal.

Penis (FM) A (FM) B

Left Testis (FM) A (FM) B

Right Testis (FM) A (FM) B

FEMALE REPRODUCTIVE TRACT

NSF / NE / NA

VULVA: NSF / NE other: _____

VAGINA: NSF / NE enlarged, hemorrhagic, purulent fluid (pus), mass, mucus, plaques, other: _____

UTERUS: NSF / NE enlarged, hemorrhagic, purulent fluid (pus), mass, mucus, plaques, other: _____

PREGNANT: Y / N Field number of fetus: _____ (see instructions below)

CERVIX: NSF / NE enlarged, hemorrhagic, purulent fluid (pus), mass, mucus, plaques, other: _____

OVARIES:

Left: NSF / NE enlarged, shrunken, mass, cyst, corpora lutea (present / absent), follicles (present / absent), other: _____

Right: NSF / NE enlarged, shrunken, mass, cyst, corpora lutea (present / absent), follicles (present / absent), other: _____

Female Reproductive Tract (FM) A (FM) B

For pregnant females, aborted fetuses, or perinatal pup deaths, examine and collect umbilicus, placenta and fetus:

UMBILICUS (describe): _____

Umbilicus (FM) A (FM) B

Sp # _____ Umbilicus (BI)

PLACENTA: Collect four 5cm x 1cm **full thickness** strips (extending through to include both the fetal and maternal side) representative of normal and any abnormal portions of the placenta. Fix one sample in each Tissue set and freeze the other two samples.

Placenta (FM) A (FM) B Sp # _____ A Placenta (BI)

B Placenta (BI)

FETUS:

A fetus or premature pup "P0" is defined as <75cm straight length; the pelage, whiskers, nails, or oral cavity not fully developed.

Complete a new Necropsy Report Form. Fetus necropsied: Y / N Necropsy # _____ (PIFSC assigns)

If complete necropsy is performed, use separate Necropsy Report Form.

Straight length: _____ cm

Axillary girth: _____ cm

Mass: _____ kg
Sex: M or F
Condition (Describe): _____

Take one swab from both the throat and rectum before beginning necropsy. If fetus is fresh, collect fluid from the stomach and freeze.

Sp # _____ Throat Swab (BI)

Sp # _____ Rectal Swab (BI)

Sp # _____ Stomach Fluid (BI)

Swabs are collected in an attempt to identify any bacterial/viral related to abortion. A swab of secondary bacterial overgrowth is not useful. If the carcass appears fresh (absence of autolysis, maggots, etc.) collect bacteriology swabs.

SPINAL CORD	NSF / NE / NA
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Using a Sawzall, cut through the ventral vertebral column through to the epaxial muscles, transecting the vertebrae, exposing intervertebral disks and spaces, spinal column, and spinal cord (medulla spinalis). Inspect all vertebral joints for abnormalities and collect 2 sets of spinal cord tissue (3-5 cm long) from the upper, middle, and lower spinal cord.

Spinal cord (FM) A (FM) B Sp # _____ A Spinal cord (BI)
B Spinal cord (BI)

BRAIN	NSF / NE / NA
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Collect the brain even if it has liquefied. Before cutting the skull, examine it carefully and photograph any fractures or blunt injuries. When opening the skull to collect the brain, collect the injured portions of the skull without damaging them. To prevent tissue from clogging the teeth on the saw, first clean away any tissue on the skull where the hacksaw blade will be cutting. Attempt to remove the brain intact and handle gently.

CEREBRUM: NSF / NE Congested, abscess, pus, hemorrhage, asymmetrical, edema, other: _____

CEREBELLUM: NSF / NE Congested, abscess, pus, hemorrhage, asymmetrical, edema, other: _____

Collect a 2x2x1cm sample from the cerebrum, cerebellum and brainstem in each Tissue Set. If any region appears abnormal, place a sample of the abnormal region in Tissue Set A as well.

Remaining portions should be wrapped in foil then frozen, same specimen # as #2, sub-number B

Brain (FM) A (FM) B Sp # _____ A Brain (BI)
B Brain (BI)

If the brain has decomposed to the point that it has liquefied, instead of collecting the above samples, collect brain in a whirlpak or cryovials and freeze.

Liquefied Brain Sp# _____ A (BI)

DURA MATER and SKULL	NSF / NE / NA
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Examine the skull for any fractures and thoroughly photograph any that are found. Examine the inside of the skull (the side against the brain). Collect any injured portions of the skull.

SKULL: discolored (describe: _____), pus, hemorrhage, congested, other: _____

The mandible (lower jaw) and all teeth within it should be collected and placed in a whirlpak for unknown age animals only. Sp

_____ Skeletal (**circle one:** injured skull/ mandible) (BI)

File No. 16632 Appendix G: Non-target Species

Non-target NMFS Species

Spinner Dolphin

Spinner dolphins (*Stenella longirostris*) occur in the Hawaiian Archipelago and may be affected by the proposed activities. The spinner dolphin is not listed as threatened or endangered under the ESA and is not listed as depleted or a strategic stock under the MMPA. Under CITES, spinner dolphins are listed on Appendix II and under the IUCN as low risk.

Spinner dolphins that may be affected by the proposed action are part of the Hawaiian Islands complex stock, and are referable to the subspecies *S. longirostris longirostris*. In the NWHI, atoll-associated communities at Kure Atoll range from 120-180 individuals; at Midway Atoll from 260-320 individuals; and at Pearl and Hermes reef approximately 350-450 individuals (L. Karczmarski, pers. comm.).

Up to 500 spinner dolphins may be harassed annually during boat transits within the lagoon waters at four NWHI sites (Midway Atoll, Pearl and Hermes Reef, Kure Atoll, and French Frigate Shoals). This incidental harassment could occur at any time of year, but would predominantly be during summer months.

Bottlenose Dolphin

Bottlenose dolphins (*Tursiops truncatus*) occur in the Hawaiian Archipelago and may be affected by the proposed activities. The Hawaiian Islands stock complex of bottlenose dolphin includes Kauai/Niihau, Oahu, 4-island, Hawaii Island, and the Hawaii Pelagic stock. This stock complex is not listed as threatened or endangered under the ESA nor depleted under the MMPA. They are listed on Appendix II under CITES and as low risk under the IUCN.

Bottlenose dolphins occur throughout the Hawaiian Archipelago in the MHI and NWHI. The abundance estimates are as follows: Kauai-Niihau – 147; Oahu – 594; 4-islands region – 153; Hawaii Island - 102; and Hawaii Pelagic (deep water and NWHI) - 3, 178 (Baird et al. 2009).

Up to 20 bottlenose dolphins may be incidentally harassed annually during boat transits in the NWHI at any time of year but predominantly during summer months.

Other Cetaceans

Humpback whales (*Megaptera novaeangliae*) mate and calve in winter months in the MHI, where aerial and vessel surveys of Hawaiian monk seals would take place, and have been observed in the NWHI, where vessels transit to deploy field camps. Humpback whales are listed as endangered under the ESA and are on CITES Appendix I. Abundance of humpback whales for the entire North Pacific Ocean is estimated to be 18,302 individuals, with over 50% of the population (approximately 10,000) estimated to winter in Hawaiian waters (Calambokidis et al. 2008). Most aerial surveys would occur

during summer months when these whales are not present, but vessel and aerial surveys and transporting seals by air and boat could occur year-round.

Other cetacean species that may be encountered near-shore in the MHI would include (in decreasing order of encounters) bottlenose dolphins (*Tursiops truncatus*), pantropical spotted dolphins (*Stenella attenuate*), and pygmy killer whales (*Feresa attenuate*). While transiting among the islands and atolls in the NWHI, cetacean species that may be encountered include humpback whales, bottlenose dolphins, pantropical spotted dolphins, Blainville's beaked whales (*Mesoplodon densirostris*), false killer whales (*Pseudorca crassidens*), sei whales (*Physeter macrocephalus*), and rough-toothed dolphins (*Steno bredanensis*) as well as numerous other cetaceans known to occur in the MHI that may also be present in the NWHI.

However, none of these cetaceans would be affected by the researchers' activities, as appropriate mitigation would be implemented to avoid harassment from aerial and vessel surveys and vessels transiting island locations. Aerial surveys would be conducted above shoreline areas. In the event cetaceans were encountered near shore, researchers would fly to an altitude of 1000 feet to avoid harassment. If encountered by boat, researchers would maintain a distance of 50 yards (150 feet) for cetaceans other than humpback whales, and a distance of 300 feet if a humpback whale is encountered. These approach distances are consistent with Federal Regulation (50 CFR 224.103) to avoid take if humpback whales are encountered and NMFS guidelines to avoid harassment of other cetaceans.

Non-target USFWS Species

ESA-listed Birds

ESA-listed bird species identified within the action area include:

- Nihoa Millerbird (*Acrocephalus familiaris kingi*),
- Nihoa finch (*Telespiza ultima*),
- Laysan finch (*Telespiza cantans*),
- Laysan duck (*Anas laysanensis*),
- Short-tailed albatross (*Phoebastria albatrus*),
- Hawaiian petrel (*Pterodroma sandwichensis*),
- Newell's shearwater (*Puffinus auricularis newelli*),
- Hawaiian stilt (*Himantopus mexicanus knudseni*), and
- Band-rumped storm petrel (*Oceanodroma castro*), a candidate species (USFWS 2010a).

No critical habitat has been designated for any of these species (USFWS 2010a).

The Nihoa finch only occurs at Nihoa Island and HMSRP researchers do not expect to encounter them. Hawaiian stilt are shorebirds that depend on large coastal wetlands and ephemeral playas in the MHI and are not likely to be encountered. Hawaiian petrel, Newell's shearwater, and band-rumped storm petrels are seabirds that nest in upper

elevation sea cliffs, and are not likely to be encountered. The following sea birds may be affected by the proposed activities.

Laysan Finch

Laysan finches are endemic to Laysan Island and were introduced to Southeast Island and Grass Island at Pearl and Hermes Reef in 1967. They are a single species and are restricted to the vegetated area of Laysan Island. Population numbers fluctuate widely, with current estimates to be 17,780 + 2819 individuals at Laysan Island and approximately 329 at Pearl and Hermes Reef (USFWS 2008a). The Laysan finch is threatened by degradation of habitat from invasive species and rising sea levels at Laysan and Pearl and Hermes Reef (Baker et al. 2006).

Both NMFS and USFWS maintain field camps at Laysan Island. NMFS also maintains field camps at Pearl and Hermes Reef. Laysan finches are tame to human presence and enter these field camps in search of food and water. A permanent field camp occupied and maintained by USFWS personnel is present at Laysan Island. It is unknown to what extent the additional presence of an HMSRP field camp (with 3 personnel) increases camp following behavior of finches. In addition, the HMSRP may erect temporary shoreline pens to hold monk seals for approximately 2 weeks at any location in the NWHI, including Laysan and Pearl and Hermes Reef.

Up to 200 individual Laysan finch may be disturbed many times during routine field camp activities each year. Laysan finches will change their behavior to search the campsite for unattended food, food scraps, or standing water. They may become more nutritionally supported than their conspecifics that do not interact with camps.

Unintentional mortality or serious injury of Laysan finches is possible. Despite efforts to prevent mortality, finches have drowned in camp containers that filled with rainwater when researchers were away from camp, or have become trapped in camp gear. Although possible, it is not expected that the finches would become entangled in shoreline net pens. Carcasses of any dead birds would be frozen and given to USFWS. Based upon past occurrences, the HMSRP expects no more than two (2) mortalities a year.

The following avoidance and minimization measures listed in the Laysan finch biological opinion (USFWS 2009) will reduce the risk of harm to the Laysan finch:

To reduce the risk of inadvertent drowning of Laysan finch at the campsite:

- Buckets will always be overturned so that they cannot collect rainwater.
- Laundry buckets must have lids while laundry is soaking.
- Water-filled buckets for dish washing (or for any other purpose) will always be attended.
- Tarps (e.g., those covering propane, etc.) will be tucked in tightly so that they cannot collect rainwater.

- Garbage cans used for desalinization will have netting placed between the can and the lid. Care will be taken to make sure the lids close properly; faulty positioning of hoses can interfere with proper closure.

To minimize accidental entanglement of Laysan finches at the campsite:

- Loose threads on fabric will be burned to minimize the risk of Laysan finch entanglement. Laysan finch feet can become entangled when fabric is hung out to dry.
- Loose threads will be cut off tents and tarps.
- Anything with small mesh (e.g., bird nets) will be put away to avoid Laysan finch entanglement.

To minimize impacts to Laysan finch from general camp activities and maintenance:

- Camp supplies and water jugs will be aligned with ample space between rows so that Laysan finches will not get trapped. Storage jugs will always be capped.
- Burn barrels will be attended at all times when burning trash. When not burning, any vents or rust-eaten holes in the barrel or lid will be covered (e.g., with rocks).
- For stability reasons, buckets will not be stacked more than two high.
- Personnel will watch for leaning buckets or water jugs and level the sand beneath leaning buckets if necessary.
- Tents will be zipped at all times (day and night) so that Laysan finches cannot enter.
- Laysan finches will not be fed or allowed access to human food. Laysan finch dependency on the camp could potentially result in adverse impacts to the Laysan finches when campsites are dismantled.
- Laysan finches appear to be limited by nest sites on the islands of Pearl and Hermes so they nest in debris (driftwood, plastic pipes, baskets, etc.). Thus, the beaches will not be cleaned or debris disturbed as this may destroy a nest. If debris poses an entanglement hazard for Hawaiian monk seals or sea turtles, it may be removed after a thorough inspection and confirmation that no Laysan finch nests are present. In an effort to prevent nesting in undesirable locations, camp gear must be checked daily during the nesting season (spring and summer) for signs that Laysan finches are building nests on or under gear. If it is determined nest building has begun, the nest site should be modified to prevent nest completion.

The Laysan finch biological opinion (USFWS 2009) provides the following reasonable and prudent measure necessary and appropriate to minimize the effect of take on Laysan finch: NMFS shall minimize the potential for harassment, harm, or mortality of Laysan finch.

In order to be exempt from the prohibitions of section 9 of the ESA, the HMSRP must follow the following terms and conditions, which carry out the reasonable and prudent measure above.

- If any unforeseen activity or action results in the harm or mortality of Laysan finches, all practicable means will be taken to apply avoidance or minimization measures to reduce the risk of additional take from that activity.
- All Laysan finch mortalities that are a result of actions which are associated with HMSRP research activities shall be reported to the USFWS within five (5) days of the incident.
- If an incidental death occurs that has not been addressed in the biological opinion, the USFWS will be contacted as soon as logistically feasible to discuss the cause of the mortality and determine the most appropriate method to avoid future mortalities from this new risk factor.
- Dead Laysan finches will be sent to Dr. Thierry M. Work at the National Wildlife Health Center, Honolulu Field Station (U.S. Geological Survey-Biological Resources Discipline) for a necropsy. The method of shipment and preservation will be determined in coordination with Dr. Work.

In addition to the measures above, personnel working in the Monument must follow terrestrial quarantine protocols for moving between islands and packing for field camps. These measures will minimize the potential for the introduction of non-native plants or insects to the Monument, which could modify habitat for Laysan finch.

Nihoa Millerbird

The Nihoa Millerbird has a small population on Nihoa Island estimated at 641 ± 295 (95% CI) (USFWS 2010a). Nihoa Millerbirds nest in small shrubs between January and May (USFWS 2010b). If not singing, they tend to stay hidden in dense vegetation, making them hard to find (USFWS 2012).

A subspecies of the Nihoa Millerbird went extinct on Laysan Island in the 1920s because of introduced grazing mammals that destroyed the birds' habitat. According to a news release (USFWS 2012), USFWS moved 24 Millerbirds from Nihoa Island to Laysan Island in September 2011, to decrease the risk of extinction from a catastrophic event on Nihoa. As of March 2012, at least 21 birds were alive, two breeding pairs were incubating eggs, and one pair was feeding nestlings. Future translocations for Millerbirds are being planned (USFWS 2012).

The Nihoa Millerbirds on Laysan Island do not show the same camp-following behavior as Laysan finch (USFWS pers. comm). Injury or death to Nihoa Millerbirds is not expected from interactions with field camps and HMSRP activities on Laysan Island. It is possible that Millerbirds could be disturbed if they nest near field camps or if HMSRP researchers hike through the interior of the island. Researchers do not expect to encounter Nihoa Millerbirds on Nihoa Island.

Short-tail Albatross

In 2008, the worldwide population of short-tailed albatross was around 2,400. There are about 450-500 breeding pairs on two islands in Japan, and the island with the majority of breeding pairs is an active volcano (USFWS 2008b).

Short-tailed albatross have been rarely seen in the NWHI at Midway Atoll (Sand and Eastern Islets), Laysan Island, French Frigate Shoals (Tern Islet), Pearl and Hermes Reef (Southeast Islet) and Kure Atoll (Green Islet). Since 1938, there have been about 50 observations of 17 individuals in the NWHI, generally between November and April (USFWS 2008b).

Midway Atoll

Short-tailed albatross typically nest on sloping grassy terraces, and a pair began nesting on Eastern Island, Midway Atoll in 2010 (USFWS 2010b). The short-tailed albatross pair on Eastern Island hatched an egg on January 14, 2011 (USFWS pers. comm.). This is the first confirmed hatching of short-tailed albatross outside of Japan in modern history (USFWS 2010b). The chick fledged in June 2011, and the breeding pair returned to the same location and successfully hatched another chick (USFWS pers. comm.).

USFWS has set up a camera to remotely view the nest and decrease disturbance from ground monitoring. When on Eastern Island, USFWS personnel maintain a distance of 150 ft (approximately 45m) from the birds, usually from behind vegetation. Any work in that area is performed when the short-tailed albatross are not present. If work must be done (e.g., camera maintenance), care is taken to decrease human visibility (USFWS pers. comm.).

The nest is located on the southeastern corner of Eastern Island, approximately 65 ft (20m) from the beach where researchers would survey monk seals. To remain out of sight of the nesting short-tailed albatross, researchers will stay low on the beach. If a researcher walks above the beach, the likelihood of being seen by the albatross is high. This could cause the chick or adults to move away from the researcher or cause the adults to fly away (USFWS pers. comm.).

Kure Atoll

Department of Land and Natural Resources (DLNR) staff have sighted short-tailed albatross on Kure Atoll from October to April in 1994, 2008, 2010, and 2011 (DLNR pers. comm. 2011). A female-female pair was observed nesting on Kure Atoll in 2010 but the egg was not fertilized. Short-tailed albatross land on Kure in the following locations:

- in camp,
- at the border of the west landfill and runway, and
- the nesting site at the southern edge of the west end of the runway.

Monk seal researchers are likely to interact with short-tailed albatross in camp and at the nest site on the west end of the runway from October to June. However, short-tailed albatross have only been sighted once in camp in January 2011 (DLNR pers. comm.).

Based on guidance from DLNR, researchers will stay out of the sight of the short-tailed albatross and keep a 500 ft (approximately 45m) buffer distance unless there is vegetation that prevents the birds from seeing humans. Researchers will take alternate paths to access the camp when short-tailed albatross are present. It is possible that a monk seal could haul up near the short-tailed albatross nesting site. If this occurred, the seal will not be approached unless there is a life-threatening situation, such as entanglement. In this case every effort will be made to minimize disturbance to the short-tailed albatross nest as required by DNLN (DLNR pers. comm.).

Laysan Island

Over the past few years on Laysan Island, a short-tailed albatross has arrived in December and is often found in the northern East Desert. NMFS researchers may encounter this bird since they tend to return from surveys through the desert. The USFWS policy on Laysan Island is to stay at least 200 ft (60m) away from the bird (USFWS pers. comm.).

Effects to short-tailed albatross

Albatross require a long straight-line ground trajectory to become airborne, and there is a small risk that they could fly into a shoreline pen fencing (erected temporarily to hold seals) with possible injury. Temporary pens had been seasonally maintained by HMSRP at Kure Atoll, Midway Atoll, and French Frigate Shoals for over ten years during summer months with no incidents of seabirds becoming entangled in the fence. However, during a 3-month maintenance of a temporary pen at French Frigate Shoals in 2006, a single Laysan albatross (*Phoebastria immutabilis*) flew into the fencing and was injured, but survived.

The HMSRP will ensure that no pens would be placed in the vicinity of short-tailed albatross or their nests. For example, at Midway Atoll, the shore pen will not be on the same island where the short-tailed albatross decoys, sound recordings, and recent nesting occurred. The placement of the pen would be on Sand Island, approximately 3 miles from the short-tailed albatross nesting location.

Monk shore pens will normally be erected in the fall, after the short-tailed albatross breeding season and fledging of hatchlings. However, pens could be erected at any time of year. If shorepens are erected, the height of the pen would be below 5 ft. HMSRP researchers would increase monitoring of pens on windy days. Pens would be dismantled immediately after use, which typically would not exceed two weeks for holding seals. In the unlikely event that a short-tailed albatross were to fly into a shorepen, the pen would be taken down and the Monument and USFWS would be contacted for guidance.

HMSRP field camps in the NWHI are typically supplied and staffed using vessels, rather than aircraft. The use of an aircraft may occasionally occur at Midway Atoll or French Frigate Shoals, which could pose a risk to short-tailed albatross. Requirements of the Monument would be in place to ensure the overall effects of air strikes on albatross and other birds is minimal (PMNM 2008). These include:

- Night flights for most of the year at Midway;
- Vegetation management along the runways to modify bird flight and nesting behavior;
- Flight path advisories given to pilots; and
- Runway clearing of birds and other wildlife by personnel prior to landing and takeoffs (PMNM 2008).

With USFWS, DLNR, and Monument mitigation measures in place to limit or eliminate interaction with short-tailed albatross, it is not likely that the activities carried out by the HMSRP would adversely affect this species. Disturbance to short-tailed albatross by HMSRP researchers will be decreased by following the required mitigation measures for each island.

Laysan Duck

The Laysan duck is found on Laysan Island and Midway Atoll. The population on Laysan is estimated at 611 (95% CI 538-714) adults (Reynolds *et al.* 2006a cited in USFWS 2009), and 200 at Midway (Reynolds *et al.* 2007a cited in USFWS 2009). The ducks use all available habitats at both locations: upland vegetation, ephemeral wetlands, freshwater seeps, mudflats, the hypersaline lake, and coastal areas (USFWS 2009).

Although these ducks primarily use vegetated upland and lake/lowland habitats, a few ducks on Laysan use the camp area to get freshwater, insects, and shade (Reynolds 2004 cited in USFWS 2009). Coastal habitats are used more frequently during the post-breeding season (September through February) than the breeding season (Reynolds 2004 cited in USFWS 2009). Flocks of up to 70 Laysan ducks were recorded on the coast during the post-breeding season (Reynolds 2004 cited in USFWS 2009).

HMSRP researchers could disturb ducks near camp. There is a small possibility that ducks in coastal areas could fly or run into the temporary monk seal holding pens when foraging. Laysan ducks have never interacted with shorepens used by the HMSRP since 1981 and any such occurrence is not expected. Thus, no injury or mortality to Laysan ducks is expected.

ESA- listed Sea Turtles

On land, sea turtles are under the jurisdiction of the USFWS and in water, under NMFS' jurisdiction.

ESA-listed sea turtle species identified within the action area include:

- Green (*Chelonia mydas*),
- Hawksbill (*Eretmochelys imbricate*),
- Loggerhead (*Caretta caretta*),
- Olive ridley (*Lepidochelys olivacea*), and
- Leatherback (*Dermochelys coriacea*).

Critical habitat has not yet been designated for any of these species in the U.S. Pacific.

Most of the sea turtle species do not occur where Hawaiian monk seals are found and would not be affected by the proposed action. None of these species (except green sea turtles) would be affected by the research activities. . Researchers do not work at night so no nesting animals would be disturbed. If turtles are seen during the day, research activities would not occur in that area. Boat drivers would watch for turtles to avoid disturbance or collision. Mitigation measures would also be carried out to avoid disturbing sea turtles.

Green sea turtles may be present on beaches where monk seal researchers conduct their work; therefore, additional detail on green sea turtles is provided below.

Green Sea Turtles

The green turtle nests in the NWHI and may be affected by the research activities when on land. The research activities will not affect green turtle hatchlings or green turtles while in the water or nesting in the MHI, as discussed below.

In Hawaii, the green sea turtle is listed as threatened under the ESA and endangered worldwide under the IUCN. Since harvest practices stopped in 1974, the Hawaiian stock of green turtles has increased and is believed to be 83% of its historical size, with an estimate of 61,000 turtles (Chaloupka and Balazs 2007).

Green turtles occur in the coastal waters surrounding the MHI throughout the year and also migrate seasonally to the NWHI to reproduce. The largest nesting colony in the central Pacific Ocean occurs at French Frigate Shoals in the NWHI, where about 200 to 700 females nest each year (Balazs 1976, as cited in Balazs and Chaloupka 2006). On occasion, green turtles also nest in the MHI. Nesting in the MHI has occurred along the north shore of Molokai, the northwest shore of Lanai, and the south, northeast, and southwest shores of Kauai.

Since harvest practices stopped in 1974, the Hawaiian stock has increased and is believed to be 83% of its historical size, giving an estimate of 61,000 for the Hawaiian stock (Chaloupka and Balazs 2007). In 2004, over 500 green turtles were recorded nesting at the East Island rookery at French Frigate Shoals (Chaloupka and Balazs 2007), where over 90% of Hawaiian green turtles nest. Research activities such as monitoring, capture, and handling of seals as well as boat landings may cause incidental disturbance to 140 basking green sea turtles a year in the NWHI, which includes French Frigate Shoals, Laysan Island, Lisianski Island, Pearl and Hermes Reef, Midway Atoll, and Kure Atoll.

Sleeping or basking green sea turtles are generally unaware of researchers that maintain a low profile such as when they are observing seals. However, some activities, such as small boat landings, capturing a seal, and other research activities may disturb basking turtles that are asleep on the beach, causing them to move into the water. Impacts to turtles are expected to be temporary, and no harm or mortality would occur. Temporary shore pens for holding monk seals would not be erected in areas where green turtles rest or nest. HMSRP researchers would monitor the pens daily for interactions with sea turtles.

Researchers work during daylight hours (sea turtles nest at night) and remain out of sight of turtles to the extent possible when carrying out activities necessary to benefit monk seals. The HMSRP does not establish field camps in the immediate vicinity of turtle nesting areas, so emerging hatchlings are not exposed to lights or disturbance.

Boats will maintain straight line paths while transiting between the islands. Boats will also avoid landing on beach areas where turtles are in the immediate vicinity. Small boats will maintain a moderate speed and watch for objects in the water including turtles to reduce the threat of boat strikes or disturbance to sea turtles in the water. Caution would be exercised in shallower waters within the atolls to avoid any disturbance to swimming green sea turtles. Therefore, takes of green sea turtles in the water are not expected.

The following conditions would be put in Permit No. 16632 based on previous consultation with USFWS:

- Walking is prohibited on all beaches, from dusk to dawn, where adult turtles rest.
- All field camps will use maximum light control (shading, minimum wattage, etc.).
- All field camps must avoid disorienting hatchling turtles.

Green sea turtles may be disturbed during some of the HMSRP activities, however they will not be harassed or harmed to the point where they would be injured or killed. The permit will require that the above measures are taken to decrease disturbance of green sea turtles. Using the best management practices for the Monument (Appendix G of Draft PEIS) would decrease potential adverse effects on turtles. These conditions for field camps and research activities in the Monument are in place to ensure preservation of the NWHI native ecosystem, including turtles (PMNM 2008).

ESA-listed Plants

ESA-listed plant species identified within the action area include:

- (*Amaranthus brownii*),
- Coastal flatsedge (*Mariscus pennatiformis*),
- Nihoa fan palm (*Pritchardia remota*),
- Nihoa carnation (*Schiedea verticillata*),
- 'Ohai (*Sesbania tomentosa*),
- 'Awiwi (*Centaurium sebaeoides*),
- Hilo ischaemum (*Ischaemum byrone*), and
- Carter's panic grass (*Panicum fauriei* var. *carteri*).

Proposed Hawaiian monk seal research and enhancement activities would have no effect on endangered plants that occur in the NWHI or MHI, as determined in 2009. The proposed activities would be located in coastal waters on the beach or within 5m inland of the splash zone. Some plants may occur on or near trail paths leading to beaches where monk seals haul out, however these plants would not be affected by research activities. Researchers primarily work on the beach or perimeter of the vegetation zone. Field

research camps in the NWHI are located further inland. The Monument requires strict quarantine procedures to avoid introducing species that might adversely affect the native biota of the NWHI.

The HMSRP would take all precautions necessary to avoid contact with ESA-listed plants including:

- Staying on the path where no vegetation occurs when accessing beaches by foot,;
- Only landing on sandy beaches below the vegetation line when accessing beaches by boat; and
- Training researchers on the identification and locations of ESA-listed plants in the MHI and NWHI.

Proposed Listed Corals

The following species of corals are proposed to be listed as threatened in the Hawaiian Archipelago:

In the MHI:

- *Montipora flabellate/M. dilatata/M. turgescens* (blue rice coral)
- *Montipora patula/M. verrilli* (spreading or sandpaper rice coral)

In the NWHI:

- *Acropora paniculata* (fuzzy table coral)

Researchers do not anchor boats in the MHI and are prohibited from anchoring boats on corals in the NWHI. It is not likely that these corals would be affected by the proposed activities.

Critical Habitat

Activities carried out by the HMSRP will not permanently alter the beach or other monk seal habitat, and will not adversely modify designated critical habitat for USFWS species. Any structures erected (e.g., tents or seal pens) would be temporary and would not be placed in areas where endangered plants are found.

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File No. 16632 Appendix I: Qualifications of Personnel

Charles Littnan, Ph.D.

Principal Investigator

Dr. Littnan is head of the Hawaiian Monk Seal Research Program of the PIFSC Protected Species Division, and was previously head of the Hawaiian Monk Seal Foraging Research Group. He has 10 years experience capturing, restraining, tagging, and attaching instruments to Hawaiian monk seals. Dr. Littnan leads the program and may execute all permitted activities excluding those procedures that must be performed by an attending veterinarian.

Selected Publications

Littnan C, Hill M, Hargrove S, Keller KE, Anders AD. 2009. Marine protected species. In: Friedlander A, Keller K, Wedding L, Clarke A, Monaco M (eds.). A marine biogeographic assessment of the Northwestern Hawaiian Islands, Chapter 6, pp. 191-234. NOAA Technical Memorandum NOS NCCOS 84. Prepared by NCCOS's Biogeography Branch in cooperation with the Office of National Marine Sanctuaries Papahānaumokuākea Marine National Monument, Silver Spring, MD. 363 p.

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Jason Baker, Ph. D.

Co-Investigator

Dr. Baker is a zoologist with the Hawaiian Monk Seal Research Program . He has 13 years experience capturing, restraining, tagging, and attaching instruments to Hawaiian monk seals, and 25 years experience conducting field research on a variety of pinniped species. Dr. Baker may execute all permitted activities excluding those procedures that must be performed by an attending veterinarian.

Selected Publications

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Michelle Barbieri, DVM, MS

Co-investigator

Dr. Barbieri is the Conservation Medicine/Hawaiian Monk Seal Health Program Coordinator. She has 15 years experience working with marine mammals including population surveys, restraint, tagging, biological sampling, health assessments, and stranding response (disentanglements, rehabilitation, surgery, and necropsies). Dr. Barbieri has performed field anesthesia for health assessments, sampling and disentanglement of Steller sea lion pups, Northern fur seals, California sea lions, Northern elephant seals, and Hawaiian monk seals. Dr. Barbieri may execute all permitted activities including those procedures that must be performed by an attending veterinarian.

Brenda Becker

Co-Investigator

Ms. Becker is a Wildlife Biologist with the Hawaiian Monk Seal Research Program. She has over 25 years experience working with Hawaiian monk seals, including extensive field presence in the NWHI observing, capturing and tagging seals. She has also assisted in maintenance and feeding of monk seals in captivity. Ms. Becker may execute all permitted activities excluding those procedures that must be performed by an attending veterinarian.

Selected Publications

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Robert Braun, DVM, MS

Co-Investigator

Dr. Braun is a veterinarian contracted to PIFSC. He has 25+ years of experience working with health issues of marine mammals. He has over 10 years experience working with Hawaiian monk seals, including sedation, physical examinations, surgeries, and oversight of captive care. Dr. Braun may execute all permitted activities including those procedures that must be performed by an attending veterinarian.

Shawn Farry

Co-Investigator

Mr. Farry is a seasonal biologist with the Hawaiian Monk Seal Research Program. He has over 10 years experience in field research teams in the NWHI, in most cases leading field teams. He has extensive experience capturing, restraining, translocating and tagging monk seals. He is a past coordinator of seal response on Kauai. Mr. Farry may execute all permitted activities excluding those procedures that must be performed by an attending veterinarian.

John Henderson, MS

Co-Investigator

Mr. Henderson is a Fisheries Biologist with the Hawaiian Monk Seal Research Program. He has worked with Hawaiian monk seals since 1981 and has extensive experience observing, restraining, and tagging Hawaiian monk seals, both in the NWHI as well as the MHI. He has also assisted in transport and captive care of Hawaiian monk seals. Mr. Henderson may execute all permitted activities excluding those procedures that must be performed by an attending veterinarian.

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Thea Johanos, MS

Co-Investigator

Ms. Johanos is a biologist with the Hawaiian Monk Seal Research Program, and has worked with Hawaiian monk seals since 1982. She has extensive experience observing, restraining, and tagging monk seals. Ms. Johanos may execute all permitted activities excluding those procedures that must be performed by an attending veterinarian.

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Angie Kaufman

Co-Investigator

Ms. Kaufman has worked first as a seasonal field biologist in 2007, and more recently as full time staff for the Hawaiian Monk Seal Research Program. She has led research field camps and has experience capturing, restraining, and tagging monk seals. Ms. Kaufman may execute all permitted activities excluding those procedures that must be performed by an attending veterinarian.

Gregg Levine, DVM

Co-Investigator

Dr. Levine is a marine mammal veterinarian contracted to the Hawaiian Monk Seal Research Program. For approximately 10 years he has sedated and health sampled monk seals both in the MHI and the NWHI and conducted surgeries. He has also conducted several necropsies on seals. Dr. Levine may execute all permitted activities including those procedures that must be performed by an attending veterinarian.

Jessica Lopez,

Co-Investigator

Ms. Lopez is a biologist with the Hawaiian Monk Seal Research Program who has worked with the program since 2003. She has led field camps in the NWHI and participated in and overseen censuses, restraint, and tagging of Hawaiian monk seals. Ms. Lopez may execute all permitted activities excluding those procedures that must be performed by an attending veterinarian.

Lizabeth Kashinsky

Co-Investigator

Ms. Kashinsky is a veterinary technician with the Health and Disease team of the Hawaiian Monk Seal Research Program. She has assisted in capture, restraint, tagging, and health sampling of Hawaiian monk seals for 10 years. She has led research field camps in the NWHI and also assisted in captive care of Hawaiian monk seals. Ms. Kashinsky may execute all permitted activities excluding those procedures that must be performed by an attending veterinarian.

Tracy Wurth

Co-Investigator

Ms Wurth is a biologist with the Hawaiian Monk Seal Research Program, having worked with the program since 2003. She presently coordinates the MHI seal sighting network. She has several years' experience leading remote NWHI field camps, and has surveyed, restrained, and tagged Hawaiian monk seals. Ms. Wurth may execute all permitted activities excluding those procedures that must be performed by an attending veterinarian.

Chad Yoshinaga

Co-Investigator

Mr. Yoshinaga has a biologist with the Hawaiian Monk Seal Research Program since 1994. He has led several field camps in the NWHI and has extensive experience capturing, handling, translocating and tagging Hawaiian monk seals. Mr. Yoshinaga may execute all permitted activities excluding those procedures that must be performed by an attending veterinarian.

File No. 16632 Appendix J: References

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