I. Application for a Permit for Scientific Research to enhance the survival or recovery of a stock under the Marine Mammal Protection Act and the Endangered Species Act.

II. Date of application: 01 December 2006

III. Applicant and Personnel

A. Applicant and Personnel

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Responsible duties:
All duties listed in permit application w. exception of leading implant surgery

Co-Investigators:

Responsible duties:
All duties listed in permit application w. exception of leading implant surgery
Dr. Jo-Ann Mellish, ASLC
Dr. Tom Gelatt, NMML / NMFS
Dr. Lorrie Rea, ADF&G
Responsible duties:
Leading and/or assisting with implant surgeries; all veterinary duties and tasks listed in permit.
Martin Haulena, DVM, Vancouver Aquarium
Pamela Tuomi, DVM, ASLC

B. Qualifications/experience of PI and CI(s)

See ***Appendix 1*** for CVs of PI and CIs.

IV. PROPOSAL

A. Summary

Using satellite-linked Life History Transmitters (LHX tags), we will estimate survival rates, and obtain long-term data on foraging effort and possible causes of mortality, in 100 juvenile (>9 mo, < 5 years) Steller sea lions (Eumetopias jubatus). We will install remote imaging systems for three-dimensional photogrammetry at locations in Alaska and Oregon, to census animals and monitor body mass, condition and health trends.
Animals will be captured from Western DPS in the northern Gulf of Alaska, and the Aleutian Islands, between July 2007 and 2012 (20-50 per year). Procedures will be

conducted under standard sterile conditions and gas anesthesia, in a transportable surgical container unit, at suitable locations aboard ships or ashore. Animals will be released after appropriate recovery periods.
We will perform comprehensive assessments of the status of body condition, health and immune system, and pollutant levels of animals prior to release. We will relate survival rates, season and causes of mortality to condition and health status, as well as seasonal, interannual and developmental dive effort. We will test the predictive power of health, condition and behavioral parameters measurable after weaning, on future survival and thus population trends.

B. Introduction

1. Species.

a. Target Species:

The target species to be taken is the Steller sea lion, *Eumetopias jubatus*, specifically the depleted (MMPA) and endangered (ESA) Western stock DPS from the northern Gulf of Alaska and Aleutian Islands regions, for the set of tasks related to the use of Life History Transmitters, and both Western DPS and Eastern DPS (threatened under ESA) for monitoring of selected rookeries via remote 3D imaging.

b. Non-Target Species:

These marine mammal species may be incidentally harassed if present at or near the work locations: California sea lion (Zalophus californianus), Harbor seal (Phoca vitulina), Northern elephant seal (Mirounga angustirostris).

Non-target avian species that may be incidentally disturbed by our research include: fork-tailed storm-petrel (*Oceanodrama furcata*), double-crested cormorant (*Phalacrocorax auritus*), pelagic cormorant (*Phalacrocorax pelagicus*), red-faced cormorant (*Phalacrocorax urile*), glaucous-winged gull (*Larus glaucescens*), black-legged kittiwake (*Rissa tridactyla*), common murre (*Uria aalge*), thick-billed murre (*Uria lomvia*), pigeon guillemot (*Cepphus columba*), parakeet auklet (*Aethia psittacula*), tufted puffin (*Fratercula cirrhata*) and horned puffin (*Fratercula corniculata*).

c. Status of species:

Target species:

Summary:

Two distinct stocks / population segments are recognized for the Steller sea lion (Eumetopias jubatus), the Western DPS and the Eastern DPS.

Last published status report: 2005.

Status of U.S. Eastern DPS: minimum population estimates: >43,700 (not corrected for animals at sea, actual MPE numbers therefore likely higher), PBR >1,900; population trends: overall stable or increasing; ESA: threatened; MMPA: depleted, strategic.

Status of U.S. Western DPS: minimum population estimates: >38,500 (not corrected for animals at sea, actual MPE numbers therefore likely higher), PBR = 231 (note that PBR management approach as originally developed is not applicable to the management of

endangered stocks); population trends: declining until 2000, increasing (overall) since 2000; ESA: endangered; MMPA: depleted, strategic.
CITES: not listed (both DPS).

Details:
Steller sea lions are found throughout the North Pacific Ocean, Bering Sea and northwest coasts of the US and Canada, reaching south to central California. Two Distinct Population Segments (DPS) have been identified within the US, the eastern and western stock / DPS, with the division at Cape Suckling, Alaska (144ºW).

Population counts of the western stock during the 1970's exceeded 190,000 individuals, of which over 160,000 occurred along the Aleutian Islands and the Western Gulf of Alaska. Recent reported counts of non-pups (= juveniles and adults) in the U.S. region of Western stock in 1996 totaled 30,622 animals; in 1998 29,257; in 2000 25,227; 26,602 in 2002. The annual rate of decline from 1991 to 2000 has averaged 4%. From 2000 to 2002, a 5.5% increase was reported for all surveyed sites of western stock. However, 2002 counts were still lower by -5.4% compared to 1998, and more than -30% compared to 1990. Total live pup counts for western stock in 35 rookeries and 10 haulouts was 8,589 pups, representing an 11.2% decline since 1998. Based on 2004 data, the current minimum population estimate for the western DPS is >38,000. The western US stock of Steller sea lions is currently listed as 'endangered' under the Endangered Species Act, and 'depleted' under the Marine Mammal Protection Act. The smaller eastern stock of the Steller sea lion, however, has not displayed an overall decrease, but instead an overall increase since the 70's. Counts of non-pups and juveniles of eastern stock totaled 21,864 in 1998, compared to 18,754 in 1992, and 15,214 in 1982. The current total minimum estimate (2004 data) is > 43,000. The Eastern stock of the Steller sea lion is currently listed as 'threatened' under the Endangered Species Act, and 'depleted' under the Marine Mammal Protection Act. (Data cited from: Sease and Gudmundson 2002, Ferrero et al. 2000, Sease et al. 2001, Angliss et al. 2001, NMFS Stock Assessment 2000).

As of 2004, counts of non-pup Steller sea lions in the Western DPS increased by up to 12% from 2000, at selected trend sites only, but these increases were not evenly distributed across the Western DPS range (Fritz and Stinchcomb 2005). Local increases as high as 20% were observed. This and other data suggest that the Western DPS Steller stock may be "oscillating around a new lower mean level" (SSL Recovery Plan, NMFS 1992, 2006).

Definitive causes for the decline are unknown, although interactions and /or competition with fisheries, predation, disease and environmental change are amongst suggested sources (SSL Recovery Plan; NMFS 1992, 2006).

Non-target species:
California sea lion: last published status report 2003, minimum population estimate >135,000, PBR >8,300; population trends increasing at >5% p.a.; ESA: not listed; MMPA: not listed, not strategic.
CITES: not listed.

Harbor seal: last published status report CA/OR/WA: 2005, AK: 1998,

CA/OR/WA: minimum population estimates: CA >31,000, PBR >1,890; population trends: possibly near carrying capacity as population growth has slowed or stopped. ESA: not listed; MMPA: not listed, not strategic.
SE Alaska: minimum population estimates: >35,000, PBR >2,100; population trends: increasing, but details are unclear since no recent status report has been published. ESA: not listed; MMPA: not listed, not strategic.
Gulf of AK: minimum population estimates: >28,900, PBR = 868; population trends: declining, but details are unclear since no recent status report has been published. ESA: not listed; MMPA: not listed, not strategic.
Bering Sea: minimum population estimates: >12,600, PBR = 868; population trends: stable or possibly declining, but details are unclear since no recent status report has been published. ESA: not listed; MMPA: not listed, not strategic.
CITES: not listed.

Northern elephant seal: last published status report 2002, minimum population estimate CA-breeding >60,500, PBR >2,500; population trends increasing; ESA: not listed; MMPA: not listed, not strategic.
CITES: LR/lc (Lower Risk – least concern)

None of the non-target avian species are listed under the ESA or Convention on International Trade in Endangered Species.

2. Background/Literature Review

The Problem:

One of the leading hypotheses for the cause of the Steller sea lion decline is a reduction in juvenile survival of 10-20% (York 1994). While Steller sea lion pups have been investigated for a number of health indices, including mass (Merrick et al. 1995), body condition (Castellini et al. 1993), morphometrics, skin-fold thickness (Jonker and Trites 2000), hematology (Rea et al. 1998), clinical chemistry (Castellini et al. 1993), immuno-competence (Zenteno-Savin et al. 1997) and pollutant levels (Lee et al. 1996; Saeki et al. 2001), there is no significant difference between 'stable' and 'declining' populations – the classic experimental design to test for effects of proximate factors on population trends - to account for the magnitude of the differences in population trends. Thus, the classic experimental design often referred to as the 'regional comparison', has not resulted in any conclusive evidence as to primary, proximate factors contributing to the Steller sea lion decline, or failure of the Western DPS to recover. Statistically, the regional comparison design is based on the differential likelihood of sampling 'problem animals' versus non-problem animals in the two regions. This likelihood may only differ by as little as 5% (the difference in population trends) or even less. This suggests more appropriate experimental designs that are based on directly identifying 'problem animals' (those afflicted / most affected by proximate factors) irrespective of region / DPS. Such experimental designs should then focus on regions of maximal likelihood of identifying 'problem animals', the Western DPS.

Furthermore, it is important to conduct an investigation geared toward the age class thought to be most at risk based on high mortality rates – juveniles (1-4 years).

Therefore, a number of physiological and morphometric indices will be examined in relation to mortality of individual juvenile animals, including hematology, clinical chemistry, viral serology, immuno-competence, pollutant levels, body condition, genetics and stress indicators. Basic hematology, clinical chemistry and stress response panels from free-range Steller sea lions will allow us to identify possible infections. Large tissue loads of toxic substances such as organochlorines can reduce the effectiveness of the immune system and contribute to reproductive failure in pinnipeds (DeLong et al. 1973, Gilmartin et al. 1976, Reijnders 1987, Hutchinson and Simmonds 1994). While these substances were not found in high levels in preliminary studies of the Steller sea lion, this is an important aspect of comparison for juvenile survivability. The measurement of body condition is an essential tool in the overall health assessment of an animal, as it can indicate levels of body energy stores. Animals with low body energy stores are less likely to endure periods of physiological and/or environmental stress. We will therefore assess the body condition (e.g. deuterium dilution, 3D photogrammetry) of study juveniles to assist in the overall assessment of health status. Blood and feces collected from free-ranging juveniles will also be analysed for evidence of stress indicators, such as cortisol or corticosterone. High levels of cortisol may indicate a physiologically stressed animal that is unable to utilize body resources in a normal fashion and may be subject to tissue wasting and eventually death. While it has been determined that two genetically distinct stocks of Steller sea lions exist in Alaska (Bickham et al. 1996, 1998) skin samples will be analysed in conjunction with NMML to expand their existing genetic databases. This information will allow us to further determine the amount of genetic diversity between and within stocks, and examine potential genetic differences between survivor and non-survivor groups. Vibrissae, hair and nail samples will be collected for stable isotope analysis to help identify the general trophic level at which an animal is feeding over prolonged periods. Specifically, stable isotope analysis will be used to determine trophic (and food web related) shifts in diet with particular interest in identifying a weaning signature. Isotopes of C and N are currently used, with possible expansion to include sulfur and oxygen as well. In addition, elemental signature analysis will be used to determine shifts in micronutrient deposition that may be related to changes in diet, and possibly getting an index of heavy metals exposure over time. These samples will be analyzed by the ADF&G as part of their ongoing stable isotope trophic level study.

Assessment of Mortality / Survival:

Mortality / survival figures are key indicators of future population trends, and are crucial data for the management of endangered species and those potentially exposed to detrimental ecological and anthropogenic environmental changes, or climate related regime shifts. In addition, data on individual survivorship is needed to assess the efficacy of programs designed to ameliorate the impact of such changes and shifts. Several life history parameters need to be accurately estimated to provide valid population models, including survival rates (preferably specific to age classes and gender), reproductive rates, and recruitment just to name the salient ones. Different techniques for collecting such vital data and monitoring populations of interest have been used historically, and new techniques are being developed. Classic techniques for monitoring survival include longitudinal mark-resight studies using permanent animal identification such as hot-

branding, or VHF-based telemetry transmitters (Burnham et al. 1987, White & Burnham 1999), or the evaluation of age structure information collected in regions/rookeries based on cross-sectional, one time samplings (Holmes & York 2003). Mark-resight studies have been used not only to assess survival rates and population trends, but also to estimate population sizes (e.g Ries et al. 1998). In fact, mark-recapture models were originally developed for abundance estimates, and not for the determination of survival rates or population trends (Schwarz 2001). Various non-VHF telemetry devices have also been used on fish (Graves et al. 2002, Goodyear 2002), and pinnipeds (McConnel et al. 2004). Early approaches to mark-resight studies required specifically structured resight efforts, whereas more recent approaches based on the Cormack-Jolly-Seber model allow the use of opportunistic resight data (see Link & Barker 2005). All of these approaches, including those using classic telemetry transmitters have to either assess or assume dispersal and emigration rates / patterns, and typically require very large sample sizes (Barker 1997, White & Burnham 1999). In addition, many approaches are analyzed based on an analysis of variance and/or linear relationships, and thus require either very large sample sizes or substantial effects to avoid incurring Type 1 errors (Gerrodette 1987).

Recently, we developed a new type of telemetry transmitter specifically designed for the collection of long-term, longitudinal data from individual animals, without any spatial constraints or resight effort (Horning & Hill 2005). These satellite-linked 'Life History Transmitters' (LHX tags) provide information on the date an animal dies, irrespective of location. Thus, these devices will provide data of a new structure, with unrestricted spatial resolution, and temporal survival resolution of 1 day. In addition, the resight effort is essentially unlimited, since each animal will be effectively monitored continuously throughout its life. As a result, certain life history parameters can be estimated with substantially smaller sample sizes, than studies based on resighting of external markings (see Horning & York, 2006).

LHX tags will provide data on survival of individual animals, and thus allow directly categorizing individual experimental animals into 'problem-' and 'non-problem' groups, leading to a new experimental design specifically tailored to the Steller sea lion problem (see 'The Problem' statement in preceding section of background information).

On a population level, survival figures are integrators over several possible proximate effects that could contribute to the population decline, such as disease and pollution, predation as well as a reduction in foraging efficiency due to changes in prey abundance and/or quality. Thus, survival figures can be utilized to monitor a population, irrespective of which proximate causes contribute (with some exceptions) to the population decline. Furthermore, mortality rates are expected to reflect nutritional stress or other proximate factors detrimental to a population several years before ultimate effects such as reduced pupping rates / pup counts become apparent (the latter presumably through a drop in recruitment).

We will specifically measure juvenile mortality, and thus directly test a crucial component of the leading hypothesis for the continuing population decline in Stellers, using implanted Life History Transmitters (LHX tags). Mortality transmitters are a well established technique to determine survival rates in wild animals. Our approach is based on a recent innovation, in that conventional mortality transmitters are externally attached and typically utilize VHF radio transmission. Several problems are associated with such devices: on pinnipeds and seabirds, external units do not remain attached beyond the

annual molt, limiting tracking to a maximum of one year. Battery-size and -capacity constraints also limit the life span of such units. Implanting mortality transmitters avoids long-term attachment problems. Implanted telemetry devices have been successfully used on a wide range of marine endotherms, and circumvent external attachment limitations. However, reception range and thus area coverage from VHF implants is reduced compared to external devices. Transmitting life span is still limited to 2-3 years.

Present State of Technology:

LHX tags were specifically developed for long-term monitoring of large marine homeotherms (Horning & Hill, 2005). The tags are described in detail in Horning & Hill 2005. The devices are implanted into the peritoneal cavity using standard sterile surgical procedures, under gas anesthesia. LHX tags monitor state and behavior of implanted animals throughout their life. After extrusion from the decomposing or partially consumed carcass, the tags float to the surface or fall out ashore, and uplink previously stored data via the ARGOS system aboard NOAA satellites. LHX tags provide data through the life of the animal on weekly cumulative foraging effort, time and date of death, as well as information on temperature gradient from two days prior, to two days following death. From this temperature data, inferences can be made on occurrence of traumatic death vs non-traumatic death. However, it is important to point out that not all deployed LHX tags will provide data, for several possible reasons. Amongst these are technical failures of the tags (although the design of the LHX tags minimizes the likelihood of technical failures compared to conventional tag designs, see Horning & Hill 2005), and situational failures (fully functional tags not being able to transmit for other reasons, e.g. being lodged under a rock, etc..). Technical LHX tag testing has demonstrated the ability of these tags to uplink while floating at sea. While tags similar in design to LHX tags – such as pop-up archival fish tags (PAT tags), have a data return rate better than 90% (R. Hill, pers. com.), the data return rate for LHX tags will likely be lower. Therefore, it becomes vital to assess the combined failure rate (technical and situational), in order to calculate a correction factor. Mortality rates are then estimated based on actual returns corrected by the determined factor. Since both technical and situational failures are likely affected by species, deployment location and other variables, the assessment of the failure rate has to be an integral part of the experimental design, and cannot be conducted on a surrogate species in a separate location. Dual LHX tag implants will be used to calculate the combined tag failure rate for this Steller sea lion application. In conjunction with carcass testing for the purpose of assessing the effects of non-independence of dual tags on failure rate determinations, this will provide correction factors required to accurately estimate survival rates. Dual tag deployment will furthermore increase the data return rate from individual animals (at least in terms of the technical failure likelihood, it is less likely that both tags inside of one animal fail, than a single one).

To date, four rehabilitated California sea lions (at The Marine Mammal Center, Sausalito, CA, NMFS permit # 1034-1685-01) and six juvenile Steller sea lions (at the ASLC, NMFS permit # 881-1668-05) have received LHX implants and have been released. Prior to release, animals were visually observed in captivity, on a daily basis, over periods ranging from four to ten weeks. All ten animals tolerated the procedures well, and showed no evidence of infections or other health complications, or of altered

behavior (Petrauskas, Mellish, Horning, unpublished data; Mellish, Horning, Tuomi and Haulena, unpublished data). At the time of release, the incision sites were well healed, based on visual inspection and the required (permit) pre-release health screening, including blood panels. All animals were monitored post-release using externally attached satellite telemetry transmitters. Post-release monitoring periods ranged from 10 days (California sea lion) to four months, likely as a function of molt-related loss of external tags. Two of the Steller sea lions were re-sighted using remote video monitoring equipment, based on brands, up to six months after release (7 months after receiving implants), and one was re-sighted nine months after release (10 months after implants). To date, no data has been returned from any of the LHX tags deployed on California sea lions and Steller sea lions, indicating that all ten animals are likely still alive. Thus, intraperitoneal implantation of dual LHX tags appears to be a viable and well tolerated procedure, as demonstrated on two species of sea lions.

Overview of implantation of telemetry devices:

Mortality transmitters have been used successfully to determine survival rates in a number of free-ranging species, and implants have been shown to be successful in other marine mammals, such as the sea otter (Monnett and Rotterman 2000). Rawson and Hartline (1964) were the first to use intraperitoneally implanted radio transmitters for the analysis of movement patterns in deer mice, in a pioneering study. Since the study of Neely and Campbell (1973), investigators have mounted efforts to assess the effects of implantation procedures and devices on the behavior and survival of implanted animals. Survival rates have been compared between externally tagged animals, and those equipped with either subcutaneous or intraperitoneal telemetry implants. The papers of Folk et al. (1971), Neely and Campbell (1973), Smith and Whitney (1977), and MacDonald and Amlaner (1980) deliver an excellent overview of the early days of the use of implantable telemetry devices in mammals (see ***Table 2*** in ***Appendix 2***). The predominant problems in early applications relate to issues of relative size, packaging and sterility of instruments and procedures. Subsequently, recommendations were made for implanted telemetry devices not to exceed 3-5% of animal body mass (MacDonald and Amlaner 1980), even though some authors later found no indications of reduced mobility with implants as large as 10% of animal body mass (Koehler et al. 1987). Modern implantable telemetry tags typically remain under 1% of body mass, a relative size considered to be unproblematic. Packaging and specifically the outermost encasing material have an effect on the likelihood of adhesion of intraperitoneal devices to intestines. This adhesion has been reported as responsible for some of the very few observed complications in intraperitoneal implants (Guynn et al. 1987). Modern inert physiologically compatible resins have resolved this issue (Monnett & Rotterman 2000, C. Monnett, pers comm.). Finally, in the early days some implantation procedures were carried out under "clean but not sterile" conditions (Eagle et al. 1984). Using appropriate instrument sterilization and sterile surgery techniques, infections from implant procedures have become virtually absent. This has reduced the incidence of post-surgical infections to 1 in 160 procedures in sea otters (C. Monnett pers comm.). In 183 yellow-bellied marmots with intraperitoneal implants, 30-day survival and growth rates, as well as pregnancy rates and mean litter sizes did not differ from controls (Van Vuren 1989). In those instances where infections occurred in earlier applications (likely as a result of

compromised sterility), bacteremia resulted in deaths within the first week after surgery, in over 90% of cases (Williams and Siniff 1983, Johnson and Berkley 1999, C. Monnett pers comm.). All studies comparing subcutaneous to intraperitoneal implantation concluded that the latter was the preferred technique, generating far fewer complications than a subcutaneous application (Neely and Campbell 1973, Philo and Follman 1981, Garshelis and Siniff 1983, Agren et al. 2000). As a result, the intraperitoneal implantation of telemetry devices into mammals in general (Marmots: Van Vuren 1989, Silver fox: Bakken et al. 1999, Badgers: Agren et al. 2000), and aquatic mammals in particular (Beavers: Wheatley 1997, Ranheim et al. 2004, River otters: Johnson and Berkley 1999, Hernandez-Divers et al. 2001, Sea otters: Monnett and Rotterman 2000), is considered a reasonably routine procedure. Amongst aquatic mammals, 6 deaths attributable to implanted devices have occurred in 410 reported procedures since 1983, and in some of these deaths occurred as a result of the use of injectable narcotics instead of gas anesthesia (Ranheim et al. 2004). Clearly, with proper sterility, gas anesthesia and careful surgical approaches deleterious effects and deaths can be minimized or avoided entirely.

The relative size of LHX tags is substantially lower than recommended limits in the case of Steller sea lions, with tags having a mass about 0.1% of animal mass.

Remote Imaging:

We have developed the **S**atellite-**Li**nked **D**ata **A**cquisition and **P**hotogrammetry System (SLiDAP System) specifically to allow year-round, semi-automated remote monitoring of sea lion rookeries in inaccessible locations, for the purpose of conducting three-dimensional photogrammetric data collection. Using visible spectrum digital still images collected via the SLiDAP system, spatially accurate 3D models of individual sea lions can be constructed, that allow the collection of accurate spatial data from individual sea lions. Data include length, girth, and volume as a proxy of body mass. We are developing models to allow the estimation / predicition of body condition through the use of multiple differential girth measurements (Mellish & Horning, in review). We are also developing enhancements to the SLiDAP system through the integration of infrared imaging capabilities. We are developing models that will further refine photogrammetric body condition estimation through the measurement of thermal patterns on individual animals (a project by M. Horning and J. Mellish, funded by the National Science Foundation of the US). We will further test the suitability of infrared imaging to assess pregnancy state of female sea lions, and general health of all animals. The SLiDAP system will furthermore facilitate obtaining frequent (daily) semi-automated census counts that will deliver census data by age class, not just by pups vs non-pups. Age classification will be conducted via length and volume estimates.

The SLiDAP system is described in Plankis et al. (in press). The application of 3D photogrammetry for the purpose of mass estimation is described in Waite (2000), Waite & Horning (2000), and Waite et al. (in press).

3. Hypothesis/Objectives and Justification

The overall objective of this research on free-ranging Steller sea lions is to aid in the investigation of the decline of the western stock and its failure to recover and to assist recovery efforts through the accumulation of essential information (including data on survival rates, seasonality of mortality events, causes of mortality, and relationship

between health and condition status and survival, as detailed below). While the application of LHX tag technology is a recent development in California sea lions and Steller sea lions, a significant aspect of the justification of this technology and particular experimental design lies with the significant reduction in the projected sample size. Due to the absence of any spatial constraints, and temporal resolution of 1 day in the LHX data set (as described above in Section 2. Background), the hypotheses listed below can be tested with a projected sample size that is lower by more than one order of magnitude, compared to conventional approaches (e.g. hot-brand based mark-resight experimental designs) (see Horning & York, 2006).
This application covers two distinct sets of tasks, through two distinct experimental approaches. One approach involves the use of animal-borne telemetry devices (LHX tags), the second involves the use of semi-automated remote imaging systems (SLiDAP System).

Task 1: LHX Tags:
We will intraperitoneally implant dual satellite-linked Life History Transmitters (LHX tags) into up to 100 free-ranging juvenile (>9 mo, < 5 years) Steller sea lions, using surgical operations under gas anesthesia conducted in a mobile unit based aboard ships or shore at suitable locations. We will perform comprehensive body condition and health assessments on all captured animals. Only animals meeting specific health and body condition criteria during the initial examination after capture will receive implants, those not meeting these criteria will be released without implants. Screening criteria are listed in Appendix 3. Implanted animals will be monitored after their release using externally attached ARGOS satellite transmitters. These external devices (Wildlife Computers SPLASH tags, or comparable devices) will transmit information on location, and dive behavior (including number, depth, duration and frequency of dives by six hourly histograms). Following extrusion from deceased animals, the LHX tags will transmit previously stored life history data via ARGOS satellites. Arrangements have been made with Service Argos for all utilized PTT ID-codes to be assigned to this project for a minimum of 15 years. A long-term service contract has been established with Service Argos.

The new experimental paradigm utilized by the life history transmitter project will allow us to directly examine the characteristics of survivors versus non-survivors. We will specifically monitor two major areas potentially responsible for a reduced juvenile population: 1) dive effort and dive behavior; and 2) body condition and health characteristics. Alterations in diving effort and activity have been linked to changes in seasonal and short-term variation in prey abundance. Dive effort data will allow for a comparison of temporal patterns of foraging effort on a weekly, seasonal and annual basis. In addition, seasonal patterns of mortality may elucidate any potential competition with major fisheries events. In this study, health and body condition assessments will serve two purposes: 1) to determine the immediate and post-operative health status of the individuals; and 2) to directly test the relationship between health status and body condition with survival versus non-survival outcomes through a multivariate approach.

We will use the collected data to:

1. Determine age specific survival rate for juvenile Steller sea lions.
2. Determine the time of year for the greatest mortality for juvenile sea lions.
3. Determine approximate locations of mortalities.
4. Analyze ontogenetic and seasonal changes in dive behavior and dive effort from deceased animals, and relate these to environmental conditions and prey abundance as assessed by other research groups.
5. Test the effects of body condition and health indicators on survival of juvenile Steller sea lions.
6. Assess the predictive power of parameters measurable in juvenile Steller sea lions for future survival.

Task 2: SLiDAP System

Using the SLiDAP imaging system we will collect frequent census information at up to 10 sites in AK (7 sites) and OR (3 sites) (frequency up to once per day). Census data will include number of animals present by age class. We will also estimate body mass and condition of individual animals. Using infrared imaging, we will attempt to assess reproductive state of females (pregnancy), and general health status, and contribute to the body condition assessment of individual animals.

We will use the images collected via the SLiDAP system to:

1. Obtain year-round frequent, accurate census data from selected study sites.
2. Assess seasonal variations in haul-out patterns and age structures of selected study rookeries.
3. Estimate longitudinal, seasonal body mass trends for clearly recognizable individuals at selected study sites, as well as cross-sectional trends for all unmarked animals.
4. Estimate longitudinal, seasonal body condition trends for clearly recognizable individuals at selected study sites, as well as cross-sectional trends for all unmarked animals.
5. Assess the relationship between observed trends in body mass and condition, and reported environmental parameters.

Recovery Actions in the Steller Sea Lion Recovery Plan addressed by this project:
The tasks outlined are in response to recommendations from several panels, including the Steller sea lion Research Peer Review (Didier 1997a, b), Steller sea lion implant workshop (Horning et al. 1999), the Steller sea lion Physiology Research Workshop Review (Williams et al. 1999), and 'Is It Food II' Workshop (DeMaster et al. 2001), and the National Marine Fisheries Steller sea lion Recovery Plan (1992, 2006).
The following is a listing of specific "Recovery Actions" for the Western DPS, listed in the 2006 revision of the Steller sea lion recovery plan (Section V: Recovery Plan for the

Western Population, Part D: Recovery Action Outline and Narrative. Listing is on pages 124-128, narrative description of recovery actions is on pages 129-156, of the SSL Recovery Plan), which our project tasks will directly or indirectly address, and a brief discussion of how these actions will be addressed. In some instances, the recovery plan suggests specific methodology for the collection of relevant data. Our project will collect some of this data using more modern and effective technology, with a smaller sample size than the suggested 'classic' methodology. Examples of modern technology replacing classic approaches are the use of LHX tags vs hot-brand-based resight studies, as well as (infrared) 3D remote imaging to obtain age specific census data, as well as health and condition assessments.

Task 1 (LHX tags) will directly address these "Recovery Actions":

1.2 Estimate vital rates
- 1.2.1 Continue to estimate survival, fecundity, and immigration/emigration rates through a branding/resight program
- 1.2.3 Develop an age-structured population model using medium format photos from aerial surveys
- 1.2.4 Develop methods and determine reproductive rates including pregnancy and parturition rates

[LHX tags will provide data on selected vital rates of SSL (some comparable to brand/re-sight data), including survival rates, and seasonal variation of survival rates. LHX tags will also form the basic platform for future technology developments and enhancements. These will likely include sensors / sensor data evaluation algorithms to allow the on-board determination, storage and transmission of data on ovulation, pregnancy and parturition]

1.3 Monitor health, body condition, and reproductive status
- 1.3.1 Examine the effects of season, age, and sex on body condition
- 1.3.2 Develop improved indices of health, body condition, and reproductive status using chemical methods (e.g., hematology serum chemistries, and endocrine monitoring)

[LHX tags and pre-deployment health assessments will provide data on health and body condition, and the relationship to survival. The assessment of the relationship between different health / condition assessment indices, and survival, will be an essential component of efforts to develop improved indices of health and condition]

2.4 Determine the environmental factors influencing sea lion foraging and survival
- 2.4.3 Distinguish how natural and anthropogenic factors influence marine ecosystem dynamics and subsequently sea lion population dynamics

[LHX tag data will be analyzed with respect to mortality events in relation to seasonality, and fishing activity. Data on individual survival and causes of mortality will substantially increase the sensitivity of any such determinations.]

4.3 Predation
- 4.3.7 Develop models to simulate predation rates based on killer whale energetics and abundance of Steller sea lion demographics

[LHX tags will provide data that will allow estimation of killer whale predation rates on instrumented animals. These can be used to test the referenced models]

5.3 Monitor causes of sea lion mortality and use data to direct management actions
[LHX tags will provide data that will allow distinction between traumatic (e.g. predation) vs non-traumatic death (e.g. starvation, disease).]

Task 1 will indirectly contribute to these actions:

2.6 Assess and protect important prey resources for sea lions
- 2.6.5 Assess the response of sea lions to changes in prey distribution and availability
- 2.6.6 Evaluate and implement appropriate fishery regulations to protect foraging habitat and prey resources for sea lions

[for both of the above listed assessments, data on survival of individual animals in relation to data on weekly cumulative foraging effort throughout the lives of the same individuals, will contribute to the assessment listed above.]

4.1 Protect Steller sea lions from disease
- 4.1.4 Evaluate causes of mortality by examining dead and live animals of all age and sex classes for disease from various sources across the geographic range and in all seasons

[All LHX implanted animals will receive health and condition assessments, which will be related to individual survival, and seasonal distribution of mortality events. Since LHX tag data will contribute to the quantification of traumatic deaths, this will help with the evaluation of other causes of mortality.]

Task 2 (SLiDAP 3D remote imaging system with infrared capability) will directly address these "Recovery Actions":

1 Baseline Population Monitoring
- 1.1 Continue to estimate population trends for pups and non-pups
  - 1.1.1 Estimate trends for pups and non-pups via aerial surveys

[at SLiDAP installation locations, census data on pups and non-pups (non-pups by age classes based on 3D photogrammetric body volume estimates) will be obtained with daily resolution, far exceeding any aerial survey or resight resolution/capability].

- 1.2 Estimate vital rates
  - 1.2.1 Continue to estimate survival, fecundity, and immigration/emigration rates through a branding/resight program

[at SLiDAP installation locations, census data on pups and non-pups (non-pups by age classes based on 3D photogrammetric body volume estimates) will be obtained with daily resolution. The SLiDAP system will allow the identification of brands with a greater temporal resolution (up to 1/day) than conventional brand resight efforts. In conjunction

with branding programs conducted by agencies, this data will contribute to the assessment of survival, fecundity, immigration and emigration rates.].

1.2.3 Develop an age-structured population model using medium format photos from aerial surveys

[at SLiDAP installation locations, census data will include information on age of individual animals present at the location, by age class, determined via 3D-photogrammetric body volume estimates. This information on the age structure at selected locations will be provided with a resolution of up to 1 day, far exceeding the temporal resolution capability of aerial surveys.]

1.2.4 Develop methods and determine reproductive rates including pregnancy and parturition rates

[at SLiDAP installation locations we will integrate infrared imaging capability into the SLiDAP system. We will develop methods to use infrared temperature data to estimate body condition and health status of individual animals, and to assess reproductive status of females.]

1.3 Monitor health, body condition, and reproductive status

1.3.1 Examine the effects of season, age, and sex on body condition

[The SLiDAP system will provide information on (volumetric) age class, health, condition and pregnancy (via infrared imaging), at installation locations.]

1.4 Develop and implement live capture methods and non-lethal sampling techniques

1.4.2 Develop improved non-lethal sampling techniques to assess health

[The SLiDAP system will provide information on body mass and body condition (estimated via 3D photogrammetric morphometric measurements), as well as infrared based health and condition assessments, in a non-invasive way, once our efforts to develop quantitative relationships between the referenced parameters are completed.]

<u>The Steller sea lion LHX project (Task 1) will directly test the following primary hypotheses:</u>
(listed as null-hypotheses)

***Ho* 1:** Juvenile survival does not differ from predicted value for constant population levels.
***Ha*:** (juvenile survival differs from predicted value) supports the conclusion that juvenile mortality (if lower) contributes to the population decline of the Western Steller sea lion stock.

***Ho* 2:** Juvenile mortality is uniformly distributed across the year.

***Ho* 3:** Dive effort does not differ between deceased animals and survivors.

***Ho* 4:** Juvenile dive effort is uniformly distributed across the year.

***Ho* 5:** Seasonal changes in dive effort - if observed (see *Ho* 4) - do not differ between animals that die at an early age, and those that survive longer.

***Ho* 6:** Juvenile survival is not related to diving propensity just after weaning.

***Ho* 7:** Juvenile survival is not related to body mass, health & condition indicators at time of LHX implantation.

***Ho* 8:** Juvenile survival is not related to levels of pollutants measured at time of LHX implantation.

The Steller sea lion SLiDAP project (Task 2) will directly test the following primary hypotheses:
(listed as null-hypotheses)

***Ho* 1:** Longitudinal and cross-sectional body mass values obtained in the rookeries observed via the SLIDAP systems do not exhibit significant seasonal variation.

***Ho* 2:** Longitudinal and cross-sectional body condition does not exhibit significant seasonal variation

***Ho* 3:** (set): Body mass and body condition trends (if observed) are not related to reported prey biomass removal values and other environmental parameters.

***Ho* 4:** There is no seasonal variation in numbers of individuals hauled out, as observed via the SLIDAP systems.

***Ho* 5:** There is no seasonal variation in the distribution of age classes present in monitored sites.

Choice of Species: This project is focused on the collection of information crucial for the ongoing Steller sea lion recovery effort. Therefore, the most accurate data will only be available through directed research on this species. As juvenile Steller sea lions are thought to be the age class which may be experiencing the largest amount of mortality, it is necessary to assess directly the characteristics of surviving individuals, compared to those that do not.

Justification of numbers:
Task 1:
The following power calculation reveals a minimum sample size of 72 dual LHX tag implanted juvenile Steller sea lions required for the assessment of age-specific juvenile annual survival rates. This calculation is based on the release of 72 animals implanted with two LHX tags each, and assuming at least 2 years of post-release monitoring.
However, this power test is a simplification, since survival between the ages of 1 and 5 is not thought to be linear, but likely increases. In addition, information from implanted animals may only be used in testing hypotheses if the animals can be confirmed via external tracking devices to have survived the first two weeks following release after

implantation. The time frame of two weeks will be used for this assessment for two reasons:

1) beyond two weeks we cannot distinguish between the status of the instrumented animal, and the status of the external tag: while external tags often last longer than two weeks, a significant percentage falls off animals after as little as two weeks.
2) Veterinarians suggest that the first two weeks after an implant surgery are the critical period: if complications arise, e.g. due to systemic infections, this will occur within two weeks (see Horning et al. 1999, Mellish et al. 2006).

Animals for which this cannot be confirmed will have to be excluded from the study. Furthermore, depending on logistical and/or permit related constraints, effects may be diluted through inclusion of seasonal or interannual variability. We are therefore requesting a maximum sample size of 100 individuals to be implanted under this permit. ***This number may include animals that may receive LHX implants under the ASLC transient juvenile sea lion program***, in other words: the sample size requested here may be partially met by animals under the ASLC Transient program if they meet the following criteria: the animals fall within the age range stipulated for this study (see Take Table), receive dual LHX tag implants using the same procedure as other LHX implanted animals, pass the health screening criteria listed in Appendix 3, are tracked following their release using external tracking devices to allow meeting the two week post-release criterion listed above, and the time frame of their release falls within the time frame of this study.

Power Test:

A power test for a one-sided analysis of variance reveals the following probabilities of committing a type II error β (not rejecting Ho when it should be rejected), for a sample size of 72 transmitter equipped animals, $\alpha=0.05$,

Ho: annual juvenile survival=0.78 (life table estimate); as well as minimum detectable decrease in survival for $\beta = 0.1$

*H*a: annual survival:	0.624	0.65	0.702	minimum detection
annual reduction in survival:	-20%	-16.7%	-10%	decrease
after one year:	$\beta=0.2$	$\beta=0.4$	$\beta>0.8$	-28%
after two years:	$\beta=0.01$	$\beta=0.02$	$\beta=0.026$	-4.2%

This calculation assumes amending mortality detections via LHX tag 'returns' by a correction factor determined to an accuracy of better than 1% from the ratio of dual versus single hits from the dual 'redundant' LHX implants. Thus, after monitoring 72 animals for >=2 years, we can detect a decrease in annual survival of as little as 4.2% with a likelihood of $\beta<0.1$ of committing a type II error. Greater reductions in annual survival will deliver significant effects at an earlier stage. Monitoring for >2 years will further increase sensitivity.

Task 2:

There are no specific sample size numbers for Task 2 (SLiDAP), since the sample size will depend on animal attendance and haul-out patterns.

C. Methods

1. Duration of Project and Location of Taking

Task 1:

The capture, biological sampling implantation and attachment of scientific instruments will take place from July 1, 2007 to June 30, 2012.

As indicated in the accompanying Take Table (Table 1), Steller sea lions will be captured from the Western DPS. The choice of which rookeries or haulouts that we capture from will be made approximately 3-6 months before the field work commences and will be based on careful consideration of the following factors: most recent population trends for that site or its region; most recent data on the prey base for the area around that site; whether that site has been disturbed or is expected to be disturbed in the near future by other researchers; and whether collaborative studies can be arranged to reduce cumulative effects of research or to capitalize on concurrent complementary Steller sea lion research or fish stock assessments at or near a site. The planning of such research will be done in consultation with Steller sea lion investigators at the National Marine Mammal Laboratory (NMFS), the Alaska Dept. of Fish & Game (ADF&G), the Alaska Sea Life Center (ASLC) and other principal investigators that hold the major Steller sea lion field research permits. In the past, the major permits were held by the Alaska Department of Fish and Game, National Marine Mammal Laboratory (NMFS) and Alaska SeaLife Center. The three Principal Investigators holding these permits, Dr. Lorrie Rea, Dr. Tom Gelatt and Mr. Don Calkins, and many of their co-investigators are in regular contact with each other and with the various co-investigators on all permits. This close contact has led to sharing logistics and personnel on a regular basis. In addition, usually one or two of the three coordinate regularly with the other Principal Investigators holding or applying for permits to conduct research on Steller sea lions. Dr. Gelatt, the National Marine Fisheries Service Permit principal investigator, regularly (at least annually) hosts coordination meetings with appropriate permit holders to help enhance synergistic effects and reduce unnecessary overlap and avoid duplicative research. Ad-hoc determination of sampling locations and dates is not a problem for our experimental design: as detailed in section B.2. (Background, 'The Problem') our experimental design with LHX tags is based on directly identifying 'problem animals' within the Western DPS using mortality information on individual animals, irrespective of small scale capture location. As the time of an impending action approaches and dates and locations are more finalized in coordination with all project principal and co-investigators and all participating organizations, this information will be provided to the NMFS Regional Coordinator. It is currently projected that initial captures and procedures will occur in the summer of 2007 between Prince William Sound and Kodiak Island, and that subsequently captures and procedures will occur in either the same region, or further West along the Aleutian Islands. Animal procedures will occur aboard ships, or ashore at suitable locations where the mobile surgical unit can be set up, based on accessibility / closeness of suitable ship anchorage or mobile unit setup sites to capture locations.

Task 2:

The installation, use and servicing of remote imaging systems will take place from July 1, 2007 to June 30, 2012. Initial installations of SLiDAP imaging systems will require site

visits over a period of up to ten days per site. This is the maximum time frame determined for a full installation and on-site calibration of the system based on the first test installation in Alaska (this first test installation was not set up near Sea lion sites), and takes into account potential difficulties including inclement weather. Installations will occur over a period of three years, starting in 2007, and through 2010, and will occur between August 1st and May 15th (outside of breeding season). Subsequently, up to two service visits per year will be required, one for annual scheduled maintenance, and up to one additional visit when failures occur. Service visits will occur between August 1st and May 15th (outside of breeding season). Operation of systems will continue until 2012 under this permit as requested here. We propose to install three systems in OR (Eastern DPS), at the two OR rookeries (Rogue Reef and Orford Reef), and one of eight known haulouts (to be determined), and seven systems in AK (Western DPS) at rookeries and haulouts to be determined. The AK installations will occur at locations in Prince William Sound, and to the West / North thereof. The criteria for site selection are: suitable topography for conducting 3D photogrammetry, and site topography suitable for landing the equipment via boat (skiff) or helicopter at a nearby location (helicopter landings not at the installation site). The determination will be made in consulation with all P.I.s and co-I's, and with P.I.s of other population monitoring projects in Western DPS region.

2. Types of Activities, Methods and Numbers of Animals or Specimens to be Taken or Imported/Exported

a. Take Tables:
Table 1a (attached) lists all Takes for Task 1 (LHX tag work).
Table 1b (attached) lists all Takes for Task 2 (SLiDAP work).

b. Description of Activities to be Conducted:

Task 1, LHX tags:
Target age group, determination of age of animals and sample sizes – The target age group (most desirable age group) for the Life History Transmitter implants is restricted to weaned animals between the ages of 12 and 28 months. It is important for the project to attempt to deploy as many LHX transmitters as close to weaning of animals as possible. However, due to difficulties in accurately determining the age of young sea lions and their weaning status, we propose to work within the effective age range of 9 months to 4 years. We propose this age range because within this age range we will be able to accomplish the proposed goals of the LHX project, and because the actual health and body condition of the subject animals is likely a better criterion to select animals for the purpose of minimizing deleterious effects on individuals and the population, than a more or less arbitrary age criterion, or a criterion based on assumed weaning, which is inherently difficult to establish. In addition, due to difficulties in accurately establishing the age of young see lions (see next paragraph), the variability in canine length and body mass for such animals does not allow for a clear distinction between 9 months and 12 months of age. For example, the smallest canine length of known age 12-month old animals was at the level of the regression fit for 9 month old animals (King et al., 2004,

cited with permission). Even greater variability can occur in body mass to age regressions. The same difficulty applies to the distinction of older animals. With a lower end of the targeted age class of 12 months, we are therefore requesting authorization to work with ages as young as 9 months, to avoid possible permit violations that may result from natural variability in age determination criteria, and an upper limit of 4 years, while we will make every attempt possible to primarily work with animals of an age between 12 and 28 months.

It is important to note that age of young Steller sea lions cannot be accurately determined unless animals have been previously captured and tagged, marked or branded in a still recognizable manner. In the absence of such markings, the best available procedure to date to estimate the age of young Steller sea lions is based on the measurement of canine length (King et al. 2004), which cannot be done until the animal has been captured, brought on board the support vessel, masked for induction of gas anesthesia, anesthetized and intubated. While every effort will be made to avoid the capture of animals that appear to be younger than 9 months, occasional capture of such young animals cannot be avoided. When such young animals are accidentally captured and age based on canine length is estimated to be likely less than 9 months, these animals will be sampled in identical manner to older animals, but will not receive LHX tag implants. There are several good reasons for proceeding with such sampling on younger animals: 1) the animal has already been subjected to the stress associated with capture, restraint and anesthesia, and 2) age estimation based on canine length is done with an as yet experimentally undetermined degree of uncertainty. As a result there may be a significant sampling bias or artifact associated with only sampling animals thought to be older based on an unverified method. Sampling of such animals of questionable age may help identify additional aging criteria. Animals that are within the permissible age range for our study of 9 months to 4 years may then be selected for LHX tag implantation, depending on the progress of captures and the study, and previously achieved sample sizes. The desired minimum sample size for this study is 72 LHX tag implanted animals. However, only a subset of the captured animals will pass initial health exams (Appendix 3) to allow implantation, and only animals that can be monitored via external satellite transmitters for a minimum of two weeks after their release, may be used for data analysis within this study. As a result, we propose to capture up to 140 animals and implant up to 100 animals over the course of likely two to three, but maximally five years.

Capture/restraint - We will capture up to 140 Steller sea lion juveniles at any time of the year and at locations described above under section C.1., by these methods that are standard procedure by the National Marine Mammal Lab, Alaska Department of Fish and Game, and ASLC for animals of this age class (Merrick et al. 1997; McAllister et al. 1997, Mellish et al. 2006): underwater capture via lasso by a dive team (McAllister et al. 1997, Mellish et al. 2006), on land using hoop nets (Merrick et al. 1997), and via platform. Locations of capture are listed in Section C.1. The decision as to which capture method to use will be based on the behavior of the sea lions, their location, the topography of the substrate when they are on land or the sea state when they are in the water. Whether underwater or on land, the lasso will be held on the end of a pole and placed around a sea lion, slightly anterior to the fore flippers. The lasso will be tightened as the sea lion moves away from the capturer, and the rope is then retrieved by personnel

in a waiting skiff. Animals are wrapped in a restraining net and pulled into the skiff directly into a capture cage.

The platform method employs a 12-ft. wide buoy with a 12-ft. by 12-ft. platform for a haul-out surface. There are 6-ft. high steel cage walls around the perimeter of the platform, with a wide trap door on one side. Sea lions haul out and return to the water freely through the trap door. To capture sea lions, the trap door is dropped when sea lions are hauled out inside. Captured sea lions are transferred into an adjacent holding cage brought up by a 30-foot barge that docks with the capture cage one at a time, by opening adjacent doors in trap and cage, then moved one at a time from the holding cage into an adjacent stainless steel squeeze cage by opening both adjacent doors simultaneously. This system is analogous to handling runs and squeeze cages used for livestock on farms and ranches. Multiple animals may be captured in the platform cage, including animals of an age outside of the target age. As a function of the size of the platform cage, up to 10 animals may be captured at one time. The animals in the trap cage will be monitored visually. The squeeze cage restricts the movement of the sea lions without harming them or exposing handlers to unnecessary risk. While an individual sea lion is in the squeeze cage, we can weigh and measure it, draw blood, and collect other biological samples. Blood samples are analyzed on site for immediate health screening. Animals outside of the target age range, or animals that are disqualified from implant surgery (see Appendix 3) will be released from the squeeze cage immediately after sampling, at the trap location. The squeeze is opened, the animal is either released if not qualified for implant surgery, by opening one end of the squeeze cage, allowing direct access to water, or moved into a separate container for transportation to the mobile surgical unit by placing the transportation container next to the squeeze cage and opening both adjacent doors simultaneously. If the mobile surgical unit is set up on the same barge or an adjacent barge (see description of mobile unit and setup locations below), a capture container will be used instead of the transport container, to move the animal into the nearby surgical unit. Animals that are not qualified and released will not be monitored after release. If gas anesthesia is used (see next), then animals will be allowed to fully recover following anesthesia before relase. The next animal is then allowed into the squeeze cage.

Gas anesthesia will be administered to facilitate sampling procedures, using standard gas anesthesia protocols. For isoflurane inhalant gas anesthesia, animals that can be positioned in sternal recumbency and restrained by use of the squeeze cage bars or a capture box will be masked using a modified traffic cone held tightly over the face to create an air seal. Additional restraint of the head will be achieved as required (as determined by the professional conducting the anesthesia) by using a head board and by placing bumper pads on either side of the head under the squeeze bars to assist in positioning nose forward for placement of the mask. Animals that cannot be adequately restrained for mask induction as determined by professional conducting the anesthesia will be premedicated with intramuscular injections of atropine (0.54 mg/ml) dosed at 0.03 ml /kg and followed at least 10 minutes later by Telazol (tiletamine HCl and zolazepam HCl) at a dose of 0.5 -2 mg/kg. Midazolam may be used as an alternate drug for sedation at a dose of 0.2 mg/kg IM. This dose does not immobilize but does appear to reduce anxiety and resistance to manipulation therefore allowing use of the squeeze cage restraint for administration of the isoflourane mask induction. It is possible to reverse some effects of midazolam with an injection of flumazenil (1 ml / 10-15 mg midazolam

IV or IM). Sedation/preanaesthetic drugs will be administered intramuscularly by jab pole syringe. Isoflurane gas will be administered for induction at 5% in medical oxygen with a flow rate of 5 to 10 liters per minute from a properly cleaned and calibrated vaporizer in a closed circuit gas anesthesia machine via the cone mask. Depth of anesthesia will be judged by the anaesthetist based on respiratory rate and volume, response to stimuli, palpebral reflex, capillary refill, and jaw and muscle tone, and maintained using 1% to 3% isoflurane in 5-10 liters of oxygen per minute flow rate as needed. Additional restraint may be achieved by using nylon straps over the body of the sea lion. Intubation with an appropriate sized (10-16 mm) cuffed endotracheal tube should be utilized for continued administration of isoflurane gas and oxygen whenever possible but may be omitted for very short procedures (less than 10 minutes) or when otherwise contraindicated. Respiratory rate, and possibly heart rate, will be monitored during anesthetic procedures and recorded on a written anesthetic record sheet indicating time and duration of anesthesia, rate of isoflurane and oxygen administration, procedures performed, drugs or other products administered and reactions of the animal from induction through anesthetic recovery.

An emergency response kit (Appendix 4) will be present at all times and will contain amongst other drugs a respiratory stimulant (doxepram), a cardiac stimulant (epinephrine), a parasympatholytic agent (atropine) and a corticosteroid (dexamethasone). Positive pressure oxygen ventilation utilizing the endotracheal tube and a 1 to 5 L re-breathing bag or an assisted respiration bellows system on the anesthetic machine will also be available if needed.

Administration of isoflurane gas will be discontinued as soon as possible after the completion of necessary research procedures. Oxygen will be administered for several additional minutes until the endotracheal tube can be removed (as judged by the return of jaw tone and swallowing reflexes). The animal will be monitored by the anesthetist for vital signs and body temperature until it regains voluntary mobility and is ready for release.

Transportation to support vessel, or shore based surgical facility – Up to 100 juvenile Steller sea lions (1-4 years of age) will be captured in water or on land using standard techniques as described above. Animals will be maintained temporarily (≤ 4hrs) in capture boxes similar to those currently in use by ADF&G and NMML (e.g., dimensions approximately 69" long x 15" high x 24" wide). For non-immediate transfer via support vessel (i.e., transport time of up to 48hrs), animals will be held in specialized holding containers (7' long x 5' high x 3' wide, with appropriate ventilation gaps / holes, similar to those currently in use by ADF&G and NMML). An experienced member of the research or husbandry team will be assigned to monitor the temperature and overall environment of the transport box for the duration of the holding period. Animals will be sheltered from noise and visual disturbance as much as possible. A veterinarian will be available at the point of departure and arrival. An emergency response kit (Appendix 4) will accompany the animal/s at all times. Once aboard the large support vessel, or the shore based facility, animals will be restrained and masked for gas anesthesia prior to transfer into the surgical unit, as described under Capture/Restraint (above). If required, premedication as described above may be used.

Description of mobile surgical facility:
We will use a mobile, container-based facility currently in use by the ASLC, for conducting ship- or shore-based LHX tag implant surgeries. This facility measures approximately (WxLxH) 10ft x 16ft x 9ft. The container is made of fibreglass over a metal frame. The inside material is a resin-based paint suitable for sterilization. The container has two doors, a window, lighting, a heating and ventilation system. It has a stainless steel work bench and sink, with hookups for electricity and running water. A mobile stainless steel surgical table is used. The unit is well set up for conducting surgeries and gas anesthesia, and has been used for both purposes, both aboard ships and based on shore at the ASLC. The previous six LHX implant surgeries that have been conducted to date on Stellers were performed in this unit.
The mobile surgical unit will be set up on board of a support vessel, on a floating barge (see description of floating trap capture) or at a suitable shore based location. Shore based locations will be used, if suitable locations are available within capture support skiff travel distance from capture locations. Shore based setup locations will meet the following minimum criteria: Locations will be concrete or asphalt covered flat areas with utilities hookup. Locations will be at research, port, industrial or military facilities, where access to setup location can be restricted, so that only authorized personnel can enter, and terrestrial / aquatic animals can be kept away (e.g. dogs, sea lions, etc..). Locations will be a short distance from an unloading dock/area where the transport containers with sea lions can be unloaded from capture skiff. The restricted access area will be large enough to house the mobile unit, and up to two transport cages (described above under capture) that will be used to hold animals for observation following surgery, for periods up to 48 hours. One example of a suitable shore based location is the loading dock adjacent to the ASLC transient sea lion holding facility. The flat concrete area is protected against unauthorized and terrestrial animal access, has electric and water hookup, and has sufficient space to accommodate sea lion transport cages, and is close to a loading dock.

<u>Sampling and injections under gas anesthesia, on up to 140 juvenile Steller sea lions:</u>

Ultrasound – A portable ultrasound unit will be utilized to record blubber depth from all captured animals. Blubber will be measured from multiple sites, including the neck, shoulder region and hind quarters (Mellish et al. 2004). This procedure involves the application of water or alcohol to the fur, followed a momentary light pressure on the skin.

Injection of deuterium oxide (deuterium-labeled water) – Under anesthesia, sterile, 99.9% pure deuterium oxide (also know as deuterium-labeled water, deuterated water or heavy water) will be injected slowly IV for body composition analysis (outlined in Iverson et al. 1993, Mellish et al. 1999). Deuterium is a stable isotope of hydrogen, and deuterium oxide is physiologically equivalent to water, and equilibrates with the body water pool. Washout measurements are determined via a blood draw. Dosage will be 100mg/kg in a one-time injection after intubation, corresponding to a volume of 12-20 mL.

Blood sampling – Multiple blood samples will be collected under anesthesia. A catheter will be used when multiple samples (> 5) must be collected over time (e.g., tracer equilibration curves) in order to reduce the number of needle insertions required. One initial background sample is required prior to injection of deuterium oxide. An additional sample will be collected for the purpose of initial health screening via CBC and differential, to establish suitability of the animal for implant surgery. Subsequent to the deuterium oxide injection, blood samples will be drawn starting at 1 hour post-injection, and then at 1:30, 2:00, 2:15 and 2:30 post-injection, for a total number of eight blood samples. Blood volume collected from each animal will not exceed the lesser of: a) 1 mL per kg body mass, or b) 5% of total blood volume (based on animal mass at the time of collection, as per ASLC protocol, see MMPA #881-1443, and per Murray 2000). Blood samples will be used for these tests/assays: isotope dilution, health screening (Appendix 3) including haematology, CBC & differential, blood chemistry, microbiological and parasitological analyses, A small aliquot of blood samples (<=10mL) will be archived at the Alaska Sea Life Center, using standard tissue archiving procedures. The purpose for this archiving is to retain the ability to conduct additional analyses that may become of interest in the future in relation to data on individual animal survival from LHX tags (e.g. new tests for specific pathogen antibodies). The most common site for blood collection in SSL is the caudal gluteal vein, which is near the animal's tail, just to the side of the spine. To locate a vein, the animal must be restrained symmetrically, lying on the stomach with foreflippers tucked against the body, hindflippers straight out behind the animal. There is a small risk of infection associated with penetration of the animal's dermis by the needle. Multiple attempts to obtain a blood sample are stressful and cause some degree of pain, damage to the vein, clotting, bruising and abscess. Multiple sampling *attempts* will be limited to four *attempts* per animal, two per side.

Blubber / adipose biopsy - We will collect a blubber biopsy (up to 0.5 g) from all animals captured, for physiological and toxicological analysis. Although the most common site for blubber biopsy in Steller sea lions is at the base of the neck, or dorsal loin region, we will collect blubber samples from the site of incision for intraperitoneal LHX tag placement. This avoids the need for a second penetration of the dermis. Samples will be collected by cutting the requisite amount (up to 0.5 g) of blubber tissue with a scalpel, after the insertion of the implants, and after the innermost suture layer has been applied. This will be done to avoid washing dislodged adipose tissue cells into the peritoneal cavity. On animals that will not receive LHX tag implants (animals of improper age as determined during anesthesia, or animals with health problems that contraindicate implantation of LHX tags but permit remainder of sampling regime), a small incision (<20mm)will be made into the skin of the dorsal loin region after proper cleaning, and a blubber sample (up to 0.5 g) will be collected by way of sterile disposable biopsy punch

Skin punch – Skin samples (2 x 50 mg) will be collected from each animal captured. Skin will be collected using a 6 mm punch tool, either prior to flipper tag insertion (see above) or from the webbing between the hind flipper. There is the potential for infection at the wound site, particularly because the environment on the rookery is not aseptic and

because the activity of the animal may prolong or prevent healing by producing repetitive stress on the wound.

Skin / mucosal swabs – Swabs will be performed for multiple physiological studies. There is a very small risk of infection associated with swabbing the animal's dermis, rectum, and ocular area.

Fecal collection - Naturally excreted fecal material will be collected opportunistically for hormonal assays and parasite load assessment.

Vibrissa, hair and nail collection - One vibrissa will be pulled from each anesthetized sea lion. Pulling, rather than clipping, a vibrissa is preferable because clipping results in an unknown length remaining attached to the sea lion. Vibrissae are pulled by gripping with forceps and pulling forcefully and rapidly in one smooth motion. Hair will be clipped at the site of surgical tag implantation, under anesthesia, using electric clippers. One small portion of the tip of a flipper nail will be clipped using nail clippers. These sample collections have been included in the permit application at the request of ADF&G, for the purpose of enhancing their stable isotope trophic studies.

Flipper tag - Any animal captured will be marked with dual flexible plastic cattle ear tags (Allflex or similar type tags) for future identification, *unless the animal has been or will be branded*. Branded animals will not be additionally marked with plastic flipper tags. Plastic flipper tags are a standard type of tag and tagging is a standard procedure for the semi-permanent marking of pinnipeds (see Mellish et al. 2006, and in press) . These tags will be affixed through both fore-flippers in loose skin in the trailing edge of the flipper, near the area where the flipper meets the body. The hole is made with a tag application punch. Each animal receives two tags, one per fore-flipper, to minimize the chance of losing the ability to identify the animal should one tag be lost. These types of tags are best considered semipermanent markers as they can and do pull out because sea lions use their foreflippers in both aquatic and terrestrial locomotion. When the tag is affixed there is the potential for infection at the wound site, particularly because the environment on the rookery is not aseptic and because the activity of the animal may prolong or prevent healing by producing repetitive stress on the wound. There is also the potential for infection when a tag pulls out of the flipper, for whatever reason. In moving about on a rookery or haulout, or swimming, there is the potential for a tag to be torn out of the flipper by abrasion on the substrate or by hydrodynamic pressure. There is no quantitative information on the rate of infection caused by flipper tagging Steller sea lions.

Hot-branding –

Hot branding will be conducted as described in detail in Mellish et al. (in press). Hot irons will only be applied long enough as determined by rate of removal of the hair and outer cell layers to permanently mark the superficial dermis and not damage the underlying tissue. Brands are applied while the animal is under anesthesia in order to minimize pain. There is the potential for infection at the wound site, and the activity of the animal may prolong or prevent healing by producing repetitive stress on the wound. However, individuals typically appear to respond well to the procedure and show no

clinical signs of infection or inflammation within weeks (Mellish et al. in press). Other agencies currently branding animals in Alaska (pups and juveniles) are the National Marine Mammal Laboratory, WA and the Alaska Department of Fish and Game. Concerted efforts for brand-resight information occur with these two agencies to monitor survival, natality rates and age at first reproduction. Given the increased amount of scientific activity with Steller sea lions, it is imperative to know if an animal has been captured previously in order to obtain the maximum amount of information in the long-term for each animal.

Hot branding the sea lions that will be handled for this project has been included in this permit application at the request of the ADF&G, and the NMML / NMFS. Veterinarians participating in the LHX project have assured us that hot-branding – if properly conducted – should not affect recovery of the animals from the implant surgery, or vice versa. However, the P.I. and co-I's of the LHX project will re-evaluate the most recent publications and findings on the effects of hot-branding on wound healing and survival of marked animals, and will re-assess the suitability of branding at the time of the capture trips. If information available at that time indicates possible complications resulting from hot-branding, or a possible effect of hot-branding on survival, a decision will be made to hot-brand only ½ of all LHX-implanted animals. The purpose of such a decision – if made – will be to allow an assessment of the effect of hot-branding on survival as determined via LHX tags, and also to allow a comparison of survival between LHX implanted and non-implanted, branded animals. Non-branded animals will receive dual plastic flipper tags instead. The two possible marking methods will not be used simultaneously on the same individuals. Irrespective of the decisions that will be made at the time field work commences, all project participants support the general approach of hot-branding all animals that are handled under gas anesthesia for the purpose of facilitation other vital studies, and as recommended by all Steller sea lion research reviews and recovery plans.

Morphometrics and 3D photogrammetry – Standard Morphometric measurements will be taken, including mass, standard and curvi-linear length, and axillary girth. These procedures will be done under anesthesia and therefore should have no adverse effects on the individual. 3D Photogrammetry will be performed (Waite 2000, Waite & Horning 2000, Waite et al in review), with simultaneous images collected from digital cameras operated at four points around the animal.

Radiographic Imaging - A mobile x-ray unit may be used to examine sea lions under gas anesthesia, including all extremities, the head and neck, chest, abdomen (anterior and mid) and the pelvis as deemed necessary for diagnostic purposes (e.g. to determine bone- or internal injuries, or other health problems potentially disqualifying animals from LHX implants, if suspected) by the attending veterinarian and Principal Investigator.

Attachment of scientific instruments – One external satellite-linked dive recorder (Wildlife Computers SPLASH tags or comparable device, dimensions 10 x 4 x 4 cm), Wildlife Computers, Redmond, WA) will be attached via 5-minute or 10-minute epoxy (based on ambient temperatures) or neoprene rubber cement to the fur, along the dorsal midline above the shoulders, to all animals with LHX tag implants, and thus to a

maximum of 100 juvenile sea lions. The tags are first attached to nylon webbing pad of approximately 12 by 18 cm area, to provide a broader base for glueing to the fur, to distribute forces and enhance tag retention. This is a standard technique in participating laboratories that has been successfully applied to many pinnipeds. These external devices will not be recovered. Instead, they fall off either after the attachment has sufficiently degraded through exposure to ambient UV and saltwater, or after the annual molt, whichever happens first.

LHX life history transmitter implantation - Implantation of dual life history transmitters will be performed on up to 100 juvenile sea lions, with a minimum of three people: a surgeon, an anaesthetist and a non sterile surgical assistant. The LHX transmitters are described in detail in Horning & Hill 2005. In brief, the devices measure 42mm (dia) by 125mm (length), have a mass of less than 120g, are positively buoyant, coated entirely in resin certified to USP Class VI standards for compatibility with implantation , and gas-sterilized using Ethylene Oxide. Standard aseptic surgical technique will be practiced, including an appropriate cap and mask and a sterile barrier surgical gown and gloves. The following includes the current protocol for the surgery, however, this method may be modified and/or refined to further minimize the effects on the animal. The surgical site will be prepared by clipping hair, skin disinfection and the use of a sterile drape. The transmitters will be gas-sterilized utilizing ethylene oxide gas (EO) in suitable packaging permeable to gas but not to bacteria. Gas sterilized transmitters should be allowed to outgas for a period of 24 hours before implantation. Surgical instruments and moisture barrier surgical drapes will be purchased pre-sterilized or thoroughly washed, dried, packaged and sterilized in an autoclave or using EO gas. The animal will be positioned securely on the surgical table in dorsal recumbency. A warm water flow under the table and thermal insulating pads covering the surgical table will retard heat loss. Anesthesia will be monitored by use of a respiratory or cardiac monitor. The surgical site will be between the caudal sternum manubrium and the pubic bones, palpated through the abdominal wall. An area - 8 to 10 cm long and 4 cm on either side of the midline should be clipped and hair removed. The skin will be repeatedly scrubbed with alcohol (90% isopropyl) alternated with providone iodine on clean gauze sponges. A nonporous sterile fenestrated drape will be placed over the surgical site and held with towel clamps. The skin will be incised along the ventral midline, the subcutaneous layer and blubber are sharp dissected. The *linea alba* will be lifted with forceps to permit penetration of the abdominal wall with a scalpel blade. The *linea alba* is then sharp dissected with blade or scissors, avoiding the viscera, to a length sufficient to pass the transmitter body (approximately 7-8 cm). Alternately, a skin incision will be made parallel to the long axis of the body in the paralumbar fossa (ventral to the lumbosacral muscles and anterior to the origin of the sartorious muscle). The incision should be extended with sharp dissection through the subcutaneous and blubber layers and through the superficial layer of the lumbodorsal fascia. When the muscular abdominal wall (transverse abdominal muscle) is reached, the fascial layer should be incised parallel to the muscle fibers for 1 to 2 cm and blunt dissection used to enlarge the opening through the muscle and peritoneal layers sufficiently to insert first one, and then the second transmitter. The abdominal wall will be grasped on either side of the incision with tissue hooks and lifted up and laterally while the transmitter is inserted through the incision into the abdominal

cavity. Bleeding will be controlled with hemostatic forceps and ligatures of 2-0 absorbable monofilament suture or with electrocautery. The surgical incision will be closed in layers using absorbable suture in a simple interrupted or mattress pattern. The skin will be closed using a subcuticular pattern of absorbable suture and over sewn with a simple interrupted pattern of non-absorbable suture on a reverse cutting needle. The skin incision will be further secured by the application of surgical glue or staples. Oxygen supplementation will continue until the animal recovers sufficiently to allow removal of the endotracheal tube.

Release

Animals will be released to the wild at the termination of research activities at capture location (for immediate releases without LHX implantation), or at known haul-out locations, or with conspecifics within the known range for the population. Release will occur after animals have fully recovered from any anesthesia. Animals that have received LHX implants will be released after a monitoring period ranging from a minimum of 24 hours, and a maximum of 48 hours, except when complications arise. Complications are dealt with as described in the Contingency Plan:

Contingency Plan:

A contingency plan to deal with complications that may arise prior to release of an animal is attached as ***Appendix 5.***

Carcass work:

Up to 50 carcasses in sufficiently good condition (no substantial disintegration or bloating), from Steller sea lions of any age and gender that are encountered on an opportunistic basis, in Alaska, British Columbia, Washington, Oregon will be collected, and dual LHX tags will be inserted. The insertion site will be sewn or glued shut. The carcasses will then be left in place, or moved to a nearby location, or towed a short distance out to sea (less than 10 miles) and released. The purpose of these deployments of dual LHX tags on carcasses is to assess the effect of the non-independence of two paired tags on the calculation of correction factors, After partial or full decomposition of the carcasses, the LHX tags will fall out and/or float to the surface, and commence transmissions. The LHX tags will likely not be recovered.

Task 2, SLiDAP:

SLiDAP System installations:

SLiDAP systems will be installed at three locations in Oregon (Rogue Reef, Orford Reef, and one haulout to be determined) and seven in Alaska (at rookeries and haulouts to be determined, on Kodiak Island, and the Aleutian Islands; see also section C.1.). For each installation of a SLiDAP imaging systems, the selected rookery / haulout will be visited up to eight times over periods of up to ten days per site, to complete the installations. Up to 500 animals may be incidentally harassed per site and visit. Each installation will consist of up to 5 separate camera systems, each autonomously powered via a solar power system, and linked wirelessly. These will be located peripherally around locations of interest, and outside of the boundaries of rookeries and haulouts. Installations will be conducted between August 1st and May 15th (outside of the breeding season).

SLiDAP service visits:
At locations with SLiDAP installations, up to two service visits per year will be conducted: One visit will be conducted each year for annual maintenance, and one additional service visit will be scheduled if system failures occur. One service visits will consist of up to two eight hours visits over the course of a 48 hr period. Up to 200 animals may be incidentally harassed per site and visit. Service visits will not be conducted between May 15th and August 1st. To reduce costs, annual maintenance visits will be scheduled on an opportunistic basis, and will be combined with other research activities at SLiDAP locations. Service visits to address system failures cannot be scheduled in advance.

3. Additional Information for Removing Animals from the Wild into Captivity and Research or Enhancement on Captive or Rehabilitating Animals

N/A

4. Lethal take
a. There are no intentional lethal takes requested.
b. Unintentional mortality or serious injury:
There is no intentional lethal take in conjunction with any aspect of this application. We request authorization for serious injury / unintentional mortality and/or euthanasia of up to 15 animals over a five year period associated with this permit, but no more than 5 animals in a single year. If complications arise, procedures described in the Contingency Plan (Appendix 5) will be followed. Should euthanasia be necessary, it will be conducted according to the Euthanasia Protocol (Appendix 6). In the event that more than 4 animals die or have to be euthanized in a single year, we will review and re-evaluate the associated procedures.

5. Exports of Marine Mammals from the U.S.
No export of live individuals or animal parts are requested.

D. Research Effects and Mitigation Measures
1. Effects
a. Clearly indicate the known or anticipated effects (i.e., stress, pain, suffering, injury…) of each activity proposed on the target species in the application, based on published or unpublished data or information on other species.

Attachment of scientific instruments – There are no anticipated risks associated with satellite tag attachment. Only small amounts of epoxy and neoprene rubber cement are applied to the fur. Sea lions typically ignore the instruments and do not attempt to bite or rub them off, and they usually begin foraging within one day. This application of external

tracking devices is a standard technique in participating laboratories that has been successfully applied to many pinnipeds. These external devices will not be recovered. Instead, they fall off either after the attachment has sufficiently degraded through exposure to ambient UV and saltwater, or after the annual molt, whichever happens first.

Blood sample – There is a small risk of infection associated with penetration of the animal's dermis by the needle. Multiple attempts to obtain a blood sample are stressful and may cause some degree of pain, damage to the vein, clotting, bruising and abscess.

Capture/restraint/anesthesia – Capture activities can disturb non-target sea lions and can result in accidental injury of sea lions. Squeeze cage utilization restricts the movement of the sea lions without harming them or exposing handlers to unnecessary risk. Hypothermia or hyperthermia may occur during anesthesia.

Deuterium oxide dilution – The primary discomfort associated with this procedure is blood collection. There is a small risk of infection associated with penetration of the animal's dermis by the needle. Multiple attempts to obtain a blood sample are stressful and may cause some degree of pain, damage to the vein, clotting, bruising and abscess.

Digital imaging – There are no adverse effects anticipated with these procedures.

Fecal collection – There are no adverse effects anticipated with these procedures.

Flipper tagging - These types of tags are best considered semipermanent markers as they can and do pull out because sea lions use their foreflippers in both aquatic and terrestrial locomotion. When the tag is affixed there is the potential for infection at the wound site, particularly because the environment on the rookery is not aseptic and because the activity of the animal may prolong or prevent healing by producing repetitive stress on the wound. There is also the potential for infection when a tag pulls out of the flipper. In moving about on a rookery or haulout, or swimming, there is the potential for a tag to be torn out of the flipper by abrasion on the substrate or by hydrodynamic pressure. There is no quantitative information on the rate of infection caused by flipper tagging Steller sea lions.

Hair clipping – There are no adverse effects anticipated with these procedures.

Hot-brand – Brands are applied while the animal is under anesthesia in order to minimize pain. There is the potential for infection at the wound site, and the activity of the animal may prolong or prevent healing by producing repetitive stress on the wound. However, individuals typically appear to respond well to the procedure and show no clinical signs of infection or inflammation within weeks (Mellish et al. in press). There are two different branding techniques that provide permanent, uniquely identifiable, large marks: freeze-branding and hot branding. Freeze branding can be used to produce two different types of marks. Short contact by the branding iron (10-20 sec. per character) destroys pigment-producing cells and results in an unpigmented brand. Prolonged contact (> 45 sec. per character) destroys both hair and the pigment-producing cells for a bald

brand. Hot branding involves brief (2-4 sec. per character) application of a red-hot iron to kill both hair follicles and pigment producing cells for a permanently bald brand.

Hot branding is equal or superior to freeze branding in several ways. First, the logistical difficulties of performing freeze branding at remote sites are daunting. The major problem is to obtain, transport, and maintain coolants in remote areas. Secondly, hot branding provides a better chance of producing permanent, readable marks. Many freeze brands are unreadable the first month, while hot brands are immediately readable (Harkonen 1987). Unpigmented brands are also difficult to read on light-colored animals. Freeze branding does not guarantee a permanent mark because melanocytes may return and cause the brand to disappear (G. Antonelis, National Marine Fisheries Service, pers. comm. 1994; Keyes and Ferrell 1979). Those using hot branding have had greater success obtaining long-term identifiable marks.

The limited literature on hot versus freeze-branding suggests that both techniques cause discomfort and stress in unanesthetized animals, and neither technique is clearly better than the other from an ethical point of view. Studies on cattle have shown that both procedures stress the subject (Lay et al. 1992 a, b, c; Schwartzkopf-Genswein et al. 1997 a, b). Freeze branding produced a protracted chronic stress, partly due to the prolonged restraint of the animal and partly due to the slow thawing of the wound. A 3-digit bald freeze brand required 3 minutes or more for application of the brand, compared to 12 seconds for a comparable hot brand (Lay et al. 1992). Schwartzkopf-Genswein et al. (1997a) found that while neither technique affected weight gain after branding, freeze-branded cattle were more difficult to handle in the 10 days after branding, suggesting that cattle experienced a lingering pain from freeze-branding. Both techniques caused a rise in blood cortisol concentrations immediately after branding, with the hot branded cattle having the highest cortisol between 20 and 40 minutes after branding, but neither technique affected post-branding pain sensitivity compared with shams (Schwartzkopf-Genswein et al. 1997b). Thus, hot branding produces a brief acute stress, and the animal recovers more quickly than with freeze branding. Preliminary results from a study of the effects of freeze and hot branding harbor seals (*Phoca vitulina*) indicated there were no differences in heart or respiration rates (W. Stobo, Bedford Inst. Ocean, pers. comm. 1993). Anesthesia should help to alleviate the short term discomfort and stress of hot branding, leading us to conclude that freeze-branding is not a suitable alternative to hot-branding in sea lions.

Biologists have been using hot branding to mark Steller sea lions in Alaska since 1975 (Calkins and Pitcher 1982; Merrick et al. 1996). Animals marked in the 1975-1976 period continued to be re-sighted through 1994, while ongoing dedicated re-sighting efforts by ADF&G, NMFS and the Alaska SeaLife Center continue to observe branded animals from subsequent year classes (from 1987 to the present) throughout Alaska and Russia. A recent study conducted at the Alaska SeaLife Center demonstrated that the inflammatory and stress response to hot-branding is minimal in Steller sea lions and recovery takes place within two weeks (Mellish et al. in press). A recent study of hot-branding in southern elephant seals demonstrated that survival estimates for hot branded seals were no lower than those for flipper-tagged seals (McMahon et al. in press), and it has been previously reported that flipper tags do not affect first year survival in monk seals (Baker and Johanas 2002). Therefore, we feel justified in concluding that hot-

branding is safe and prudent and is the best available scientific method for the long-term monitoring of Steller sea lion survival.

LHX life history transmitter implantation – There is a possibility of infection associated with the implantation procedure, including swelling, elevated temperature and loss of appetite.

Morphometrics – These procedures require the temporary restraint of an animal but otherwise have no adverse effects on the individual.

Skin/mucosal swab – There is a very small risk of infection associated with swabbing the animal's dermis, rectum and ocular area.

Skin sample collection: There is the potential for infection at the collection site, particularly because the environment on the rookery is not aseptic and because the activity of the animal may prolong or prevent healing by producing repetitive stress on the wound.

Tissue collection – There is a small risk of infection associated with penetration of the animal's dermis with a scalpel/biopsy punch tool to obtain subcutaneous blubber.

Ultrasound – No adverse reactions are anticipated to this procedure.

Vibrissa extraction – There are no adverse effects anticipated with these procedures.

Radiographic imaging – The primary discomfort associated with this procedure is immobilization, which is eliminated with the use of anesthesia.

b. Also describe any potential effects of incidental harassment or take of non-target species.

There are no anticipated effects of incidental harrassment on non-target species.

2. Measures to minimize effects

Most biological sampling events will take place with the individual under anesthesia, which greatly reduces stress to the animal while also increasing safety to both animal and handlers.

Attachment of scientific instruments mitigation - We use a slow-setting, low exothermic epoxy, so there is little chance of trauma to the skin.

Blood sampling – A maximum of three needle insertions to initially locate a vein and subsequently obtain the required blood volume will be made at any one site at any one session to reduce muscle injury and bruising and to reduce risk of introducing infection. A catheter will be used when multiple samples (> 5) must be collected over time (e.g.,

tracer equilibration curves) in order to reduce the number of needle insertions required. To reduce the risk of infection, only clean, sterile disposable needles will be used to obtain blood samples and a new needle will be used for each blood collection.

Capture/restraint/anesthesia – During anesthesia, hypothermia (deep rectal temperature < 92 ° F) will be prevented by the application of warm (100-105 °F) water bags to flippers and body, drying of the fur and covering the animal with thermal insulating blankets. Hyperthermia (deep rectal temperature > 106°F) can be controlled by wetting the flippers with cool water, applying ice or cold water packs and use of fans for increased air flow. An emergency response kit (Appendix 4) will be present at all times consisting of a respiratory stimulant (e.g., doxepram), a cardiac stimulant (e.g., epinephrine), a parasympatholytic agent (e.g., atropine) and a corticosteroid (e.g., dexamethasone). Positive pressure oxygen ventilation utilizing the endotracheal tube and a rebreathing bag or an assisted respiration bellows system on the anesthetic machine will also be available if needed. Administration of isoflurane gas will be discontinued as soon as possible after the completion of necessary research procedures. Oxygen will be administered for several additional minutes until the endotracheal tube can be removed (as judged by the return of jaw tone and swallowing reflexes). The animal will monitored by the anesthetist for vital signs and body temperature until it regains voluntary mobility.

Deuterium oxide dilution – The primary discomfort associated with this procedure is blood collection. A maximum of three needle insertions to initially locate a vein and subsequently obtain the required blood volume will be made at any one site at any one session to reduce muscle injury and bruising and to reduce risk of introducing infection. A catheter will be used when multiple samples (> 5) must be collected over time (e.g., tracer equilibration curves) in order to reduce the number of needle insertions required. To reduce the risk of infection, only clean, sterile disposable needles will be used to obtain blood samples and a new needle will be used for each blood collection.

Digital imaging – There is no mitigation required for this activity.

Fecal collection - There is no mitigation required for this activity.

Hair clipping – There is no mitigation required for this activity.

Hot-branding - Brands are applied while the animal is under anesthesia in order to minimize pain. Only clean brands heated to the appropriate temperature will be used by experienced personnel.

LHX life history transmitter implantation – An emergency kit with equipment and supplies for responding to complications or emergencies will be readily available. Any animal displaying evidence of infection (swelling, wound discharge, changes in appetite) will be treated with antibiotics or additional surgery as needed and recommended by the attending veterinarian.

Morphometrics – There is no mitigation required for this activity.

Skin/mucosal swab – To reduce the risk of infection, only clean, sterile disposable swabs will be used.

Tissue collection – To reduce the risk of infection, only clean, sterile disposable scalpel blades or biopsy punch tool will be used to obtain biopsy samples and a new scalpel blade or biopsy punch tool will be used for each biopsy site.

Ultrasound – There is no mitigation required for this activity.

Vibrissal extraction – This is conducted under anesthesia.

Radiographic imaging – The primary discomfort associated with this procedure is immobilization, which is eliminated with the use of anesthesia. There is no mitigation required for this activity.

3. Monitoring effects of activities

Indicate any post-handling or post-disturbance monitoring procedures that would be conducted to evaluate the effects of the proposed activities and/or to ensure animals have recovered.

Animals are monitored post-procedure until they have completely recovered from the anesthesia or handling procedure. All free-range animals are maintained in a cool, dry area until complete recovery from anesthesia prior to release. All implanted animals are satellite tagged to facilitate long-term monitoring. This monitoring via external tags provides information on diving (and thus foraging) activity, and location of animals, as long as the external tags remain attached (2 weeks to 4 months). Animals may be hot-branded at the request of NMFS and/or ADF&G to facilitate long term recognition of animals. The remote video system used by the ASLC provides a unique and excellent opportunity for continuous behavioral monitoring of animals following procedures in the wild and for released animals. The SLiDAP imaging system will additionally provide information on long-term survival of branded animals.

4. Alternatives

a. If the proposed activities will or may cause stress, discomfort, pain, suffering, injury or mortality, explain why there are no feasible alternative methods for obtaining the data or information being sought. Indicate the status of your IACUC review and approval.

Because the objective of this study is to determine the factors that may be limiting the recovery of Steller sea lions, we must study live Steller sea lions in their natural habitats. We will use computer modeling and laboratory simulation whenever possible, but modeling studies have already demonstrated that we do not have enough basic information on many of the parameters that are needed to accurately model the behavior, survival and reproductive performance of Steller sea lions (e.g. Matthiopolous, Thompson and Boyd 2005, personal communication; NMFS 2006). All procedures

included will only be performed under valid IACUC approvals from all participating institutions (OSU, ASLC, and possibly others). Copies of these approvals will be provided prior to any sampling event.

E. Resources Needed to Accomplish Objectives

Attach copies of any relevant formal research proposals, contracts, or letters of agreement that would demonstrate the financial or logistical resources available to the applicant to conduct and complete the proposed activities.

The research included in this application is funded via various sources, through existing grants / contracts including the Steller Sea Lion Research Initiative (NOAA #NA17FX1429, #NA17FX1430), the National Science Foundation (#480431), the North Pacific Marine Research Program, the Pollock Conservation Cooperative Research Center, Oregon State University, and the Alaska Sea Life Center (through Federal Appropriations).

F. Publication of Results

Indicate where, and if possible, when the research results would be published or otherwise made available to the public and the scientific community.

Research results will be published and made available in the appropriate internationally recognized, peer-reviewed scientific journals at the discretion of the investigators involved with the respective research programs to be implemented.

V. National Environmental Policy Act (NEPA) Considerations

1. Will your research or enhancement activity involve equipment or techniques that are new, or may be considered innovative or experimental?

No. All tasks have been performed in part or in whole previously with Steller sea lions and/or other marine mammal species.

2. Does your activity involve the collection, handling, or transport of potentially infectious agents or pathogens, and/or does your activity involve the use or transport of hazardous substances? If so, provide a description of protocols to be used to ensure human safety from injury or zoonotic disease transmission. Should be standard

Yes. The collection and transport of biological samples will only occur with proper sample identification and personal protective equipment as specified in pertinent Animal Use Permits issued under USDA jurisdiction (e.g., latex gloves, disposable sampling tools, double bagged/protected sample vials, close-toed shoes). Hazardous materials shipping will be performed according to all pertinent standards at the time of shipment, under USDOT jurisdiction.

3. If any of your activities occur in or near unique geographic areas (such as State National Parks or Wilderness Areas, Wildlife Refuges, designated Critical Habitat for endangered species, etc.) *, would any aspect of your activities impact the physical environment* (*e.g.*, anchoring vessels or buoys, erecting blinds or other structures, disrupting nesting bird habitat, etc.)*?*

This research will take place in Oregon, and throughout the northern Gulf of Alaska and Aleutian Islands. Similar research has been routinely conducted in these area for several years and does not impact the physical environment in a permanent manner.

4. Do you know if your work could affect entities listed in or eligible for listing in the National Register of Historic Places, or cause loss or destruction of scientific, cultural or historic resources (*e.g.*, archeological resources, species used for subsistence purposes, etc.)? If so, list the sites and explain how they might be affected or why they would not be affected.*?*
No such entities or resources are anticipated to be affected by this work.

5. Would your proposed activities include actions that might involve the transport of any material, biological or otherwise, from one area to another?
No, all transport of animals would occur within the indigenous area.

VI. Previous and Other Permits

A. Previous permits:
LHX tag implantation has been performed on Steller sea lions under a previous permit issued to the ASLC, #881-1668-05. The holder of that permit was Tylan Schrock, Executive Director of the ASLC, the PI was Donald Calkins, M. Horning as CI.

LHX tag implantation has been performed on California sea lions under an existing permit to M. Horning (PI and Holder), #1034-1685-01.

B. Other permits:
M. Horning is PI and Holder of one other permit issued by NMFS, OPR, for research on Weddell seals, permit # 1034-1854-00.

VII. References – Listed in Appendix 7

VIII. Certification and Signature
"I hereby certify that the foregoing information is complete, true, and correct to the best of my knowledge and belief. I understand that this information is submitted for the purpose of obtaining a permit under one or more of the following statutes and regulations promulgated thereunder, as indicated in the Section I of this application:

The Endangered Species Act of 1973 (16 U.S.C. 1531-1543) and regulations (50 CFR 222.23(b)); and/or

The Marine Mammals Protection Act of 1972 (16 U.S.C. 1361-1407) and regulations (50 CFR Part 216); and/or

The Fur Seal Act of 1966 (16 U.S.C. 1151-1175).

I also understand that any false statement may subject me to criminal penalties of 18 U.S.C. 1001, or to penalties provided under the Endangered Species Act of 1973, the Marine Mammal Protection Act of 1972, or the Fur Seal Act of 1966, whichever are applicable."

Signature of applicant _____

Date _____

Typed or Printed Name of Applicant_____

Title of Applicant_____

Table 1a. Proposed **Task 1** activities between 01 July 2007 and 30 June 2012.

Species	**Life Stage**	**Sex**	**Maximal Annual Take**	**Total Take**	**# takes per individual**	**Take Action**	**Transport**	**Location**	**Dates/ Time period**
Eumetopias jubatus	juvenile 9mo -4yr	m/f	140	140	1 ea.	Capture, restraint, transportation to surgical unit, and sampling - including anesthesia, body mass/morphometrics/ digital imaging, labeled water injection, multiple blood samplings, tissue collection (blubber, skin, vibrissae, hair, nail clipping), ultrasound imaging, external tag attachment, fecal collection, skin and mucosal swab, radiographic imaging.	From capture location to mobile surgical unit, in trans-port cages, via land or water	Resurrection Bay Prince William Sound Kodiak Island Aleutian Islands	year-round
Eumetopias jubatus	juvenile 9mo -4yr	m/f	100	100	1	Intraperitoneal implantation of two Life History Transmitters	n/a	Mobile surgical unit	year-round
Eumetopias jubatus	juvenile 9mo -4yr	m/f	100	100	1	External Transmitter attachment – of ARGOS transmitters for post release monitoring	n/a	Mobile surgical unit	year-round
Eumetopias jubatus	juvenile 9mo -4yr	m/f	140	140	1	Marking - using either dual foreflipper plastic cattle tags, or hot-branding.	n/a	Mobile surgical unit	year-round
Eumetopias jubatus	juvenile 9mo -4yr	m/f	140	140	U	Contingency plan treatments - Multiple actions may be taken per animal, as outlined in the contingency plan (***Appendix 5***) in the unlikely event of pre-release complications.	n/a	Resurrection Bay Prince William Sound Kodiak Island Aleutian Islands and mobile surgical unit	year-round
Eumetopias jubatus	all ages	m/f	1500	3000	U	Incidental disturbance	n/a	Resurrection Bay Prince William Sound Kodiak Island Aleutian Islands	year-round
Eumetopias jubatus	all ages	m/f	5	15	1	unintentional mortality, including necropsy and tissue samplings to establish possible cause of death or complications. While no mortalities are intended, we request authority for up to 5 unintentional mortalities per	n/a	Resurrection Bay Prince William Sound Kodiak Island Aleutian Islands	year-round

Species	Life Stage	Sex	Maximal Annual Take	Total Take	# takes per individual	Take Action	Transport	Location	Dates/ Time period
						year, and no more than 15 total for the entire duration of the project. This includes possible euthanasia (***Appendix 6***) if required as a result of complications prior to release of animals, as detailed in the contingency plan (***Appendix 5***). In the event of more than four unintentional mortalities in a single year, procedures will be revisited and possibly modified.			
Eumetopias jubatus	all ages	m/f	50	50	1	Opportunistic carcass collection, insertion of dual LHX tags, release of carcass	local moving / towing to deposit/release location	Anywhere in Alaska, British Columbia, Washington, Oregon	year-round
Phoca vitulina	all ages	m/f	100	200	U	Incidental disturbance	n/a	All above listed work locations	Year-round

Table 1b. Proposed **Task 2** activities between 01 July 2007 and 30 June 2012.

Species	**Life Stage**	**Sex**	**# of install-ation visits per site**	**# of service visits per site per year**	**Maximal Annual Take**	**Total Take**	**# takes per individual**	**Take Action**	**Location**	**Dates/ Time period**
Eumetopias jubatus	All ages	m/f	8 visits over one 10 day period (8 total)	n/a	1500 (approx. up to 500 per site, at up to three sites)	1500 (up to three sites over three years)	8 (one per visit)	Incidental Disturbance for Eastern DPS (Oregon). Initial installation of systems	Oregon: Rogue Reef, Orford Reef, 1 haulout TBD	Aug 1 to May 15
Eumetopias jubatus	All ages	m/f	n/a	2 visits over 2 day period, twice per year (4 total)	1200 (approx. up to 200 per site, at up to three sites, twice per year)	3600	2 (one per visit)	Incidental Disturbance for Eastern DPS (Oregon). 6-monthly service of systems beginning no earlier than 6 months following initial installation	Oregon: Rogue Reef, Orford Reef, 1 haulout TBD	Aug 1 to May 15
Eumetopias jubatus	All ages	m/f	8 visits over one 10 day period (8 total)	n/a	2000 (approx. up to 500 per site, at up to four sites per year)	3500 (up to seven sites over three year period)	8 (one per visit)	Incidental Disturbance for Western DPS (Alaska). Initial installation of systems	7 TBD locations in Alaska: Kodiak Island, Aleutian Islands	Aug 1 to May 15
Eumetopias jubatus	All ages	m/f	n/a	2 visits over 2 day period, twice per year (4 total)	2800 (approx. up to 200 per site, at up to seven sites, twice per year)	8400	2 (one per visit)	Incidental Disturbance for Western DPS (Alaska). 6-monthly service of systems beginning no earlier than 6 months following initial installation	7 TBD locations in Alaska: Kodiak Island, Aleutian Islands	Aug 1 to May 15
Phoca vitulina	All ages	m/f	n/a	n/a	150	450	U	Incidental disturbance	All above listed work locations	Aug 1 to May 15
Zalophus californianus	All ages	m/f	n/a	n/a	250	750	U	Incidental disturbance	All above listed work locations	Aug 1 to May 15

Species	Life Stage	Sex	# of install-ation visits per site	# of service visits per site per year	Maximal Annual Take	Total Take	# takes per individual	Take Action	Location	Dates/ Time period
Mirounga angustirostris	All ages	m/f	n/a	n/a	200	600	U	Incidental disturbance	All above listed work locations	Aug 1 to May 15

APPENDIX 1: CV's of PI and CIs:

MARKUS HORNING

Assistant Professor of Fisheries & Wildlife, Oregon State University

Professional Preparation:

Scripps Institution of Oceanography	Physiology	Postdoctoral Research	1992 - 1996
University of Bielefeld and Max-Planck Institute for Behavioral Physiology, Germany			
	Zoology	Doctoral Degree	1992
University of Freiburg, Germany	Biology	Diplom Degree	1988

Professional Experience / Appointments:

Assistant Professor, Dept. Fisheries & Wildlife, Oregon State University, OR (2006 —).
Adjunct Associate Professor, Dept. Marine Biology, Texas A&M University at Galveston (2006 —).
Associate Professor of Marine Sciences (Affiliate) , School of Fisheries & Ocean Sciences of the *University of Alaska Fairbanks* (1999 —).
Associate Member of the Graduate Faculty of the Wildlife & Fisheries Sciences Department, College of Agriculture & Life Sciences, *Texas A&M University College Station* (1999 —).
Director, Laboratory for Applied Biotelemetry & Biotechnology at *Texas A&M University Galveston* (1999 - 2006).
Research Scientist, *Texas A&M University at Galveston*, TX (2005 – 2006).
Affiliate Faculty Member, Marine Engineering Technology, Texas A&M University at Galveston (2004 – 2006).
Associate Research Scientist, *Texas A&M University at Galveston*, TX (1998 - 2004).
Assistant Research Scientist, *Texas A&M University at Galveston*, TX (1996 - 98).
Associate Editor for *Marine Mammal Science* (1996 - 1998).
Postdoctoral Research Physiologist at the *Scripps Institution of Oceanography* (1992-96).
Biologist, *Max-Planck-Institute for Behavioral Physiology*, Seewiesen, Germany (1990 - 92).
Research Technician, *Scripps Institution of Oceanography* (1989 - 90).
Research Technician, *Scripps Institution of Oceanography* (1980 - 82).

Research projects related to procedures pertinent to this permit application with M. Horning as Principal Investigator:

1. *Satellite-linked life-history transmitters in Steller sea lions: assessing the effects of health status, foraging ability and environmental variability on juvenile survival and population trends.* This project was initiated under funding from the North Pacific Marine Research Program (NPMRP) and the Steller Sea Lion Research Initiative (SSLRI) in 2003, and is continuing. To date, six juvenile Steller sea lions received life-history transmitter implants, and were successfully released.

2. *Monitoring post-release survival in rehabilitated California sea lions (Zalophus californianus) with intraperitoneal, satellite-linked life history transmitters.* This project was initiated under funding from the North Pacific Marine Research Program (NPMRP), and from the Pollock Conservation Cooperative Research Center (PCCRC), with work conducted at The Marine Mammal Center at Sausalito, CA, starting in 2003. To date, four rehabilitated California sea lions have received life history transmitter implants, and have been successfully released. This funding has since expired. Equipment is available for the continuation of this study.

3. *Evaluation of adrenal function in serum and feces of California sea lions (Zalophus californianus).* This project was conducted at The Marine Mammal Center, on rehabilitated California sea lions, under funding from the Alaska Sea Life Center, and from the Laboratory for

Applied Biotelemetry & Biotechnology at Texas A&M University. The study was initiated in 2004, and was completed in 2005.

Publications and presentations related to the above listed projects:

Peer reviewed journal publications:

Horning M, Hill RD (2005) Designing an archival satellite transmitter for life-long deployments on oceanic vertebrates: The Life History Transmitter. IEEE Journal of Oceanic Engineering 30 (4): 807-817.

Mellish JE, Calkins DG, Christen DR, Horning M, Rea LD, Atkinson SK (2006) Temporary Captivity as a Research Tool: Comprehensive Study of Wild Pinnipeds Under Controlled Conditions. Aquatic Mammals 32 (1): 58-65.

Technical publications / reports:

Petrauskas L, Horning M, Tuomi P, Atkinson S (2005) Non-invasive monitoring of rehabilitation procedures in California and Steller sea lions. 177 - 186. In: Synopsis of Research on Steller Sea Lions 2001-2005 (Loughlin TR, Atkinson S, Calkins DG, eds.). Alaska Sea Life Center, Seward, AK 99664, USA. 344 pp.

Conference presentations:

Haulena M, Gulland F, Lander M, Harvey JT, Horning M, Tuomi P, Butler PJ, Woakes AJ (2005) Surgical implantation of tracking and physiological monitoring instruments in pinnipeds. XVI Biennial Conference on the Biology of Marine Mammals, San Diego, CA, December 12-16 2005.

Haulena M, Gulland FMD, Horning M, Tuomi P. (2005) Intra-abdominal implantation of life history transmitters in California sea lions (Zalophus californianus). IAAAM Meetings, Seward, AK, May 14-18 2005.

Hill R, Horning M. (2005) The Life History Transmitter: a new concept for long-term monitoring of oceanic vertebrates. Biologging 2 Conference, St. Andrews, U.K., June 13-16 2005.

Petrauskas L, Atkinson S, Gulland F, Mashburn K, Mellish JE, Greig D, Horning M (2003) Validating steroid hormone assays for the determnation of stress response in California sea lions (Zalophus californianus) to varying types of surgical and non-surgical procedures. XV Biennial Conference on the Biology of Marine Mammals. Greensboro, NC, USA.

Selected additional publications

Cornick LA, Inglis SD, Willis K, Horning M (2006) Effects of increased swimming costs on foraging behavior and efficiency of captive Steller sea lions: evidence for behavioral plasticity in the recovery phase of dives. J. Exp. Mar. Biol. Ecol. 333 (2): 306-314.

Willis K, Horning M (2005) A novel approach to measuring heat flux in swimming animals. J Exp Mar Biol Ecol 315(2): 147-162.

Willis K, Horning M, Rosen DAS, Trites AW (2005) Spatial variation of heat flux in Steller sea lions: evidence for consistent avenues of heat exchange along the body trunk. J Exp Mar Biol Ecol 315(2): 163-175.

Mellish JE, Tuomi P, Horning M (2004). Assessment of ultrasound imaging as a non-invasive measure of blubber thickness in pinnipeds. J Zoo Wildl Med 35(1): 116-118.

Williams TM, Fuiman LA, Horning M, Davis RW (2004) The cost of foraging by a marine predator, the Weddell seal *Leptonychotes weddellii*: pricing by the stroke. J Exp Biol. 207: 973-982.

Cornick, L.A. and M. Horning. (2003) A test of hypotheses based on optimal foraging considerations for a diving mammal using a novel experimental approach. Can J Zool, 81: 1-9.

Davis RW, Fuiman LA, Williams TM, Horning M, Hagey W (2003) Classification of Weddell Seal Dives Based on Three-Dimensional Movements and Video Recorded Observations. Mar Ecol Prog Ser 264: 109-122.

Williams TM, Davis RW, Fuiman LA, Francis J, Le Boeuf BJ, Horning M, Calambokidis J, Croll DA (2000) Sink or Swim: Strategies for Cost-Efficient Diving by Marine Mammals. Science 288: 133-136.

Horning M, Trillmich F (1999) Lunar cycles in diel prey migrations exert stronger effect on diving of juveniles than adult Galápagos fur seals. Proc. Royal Soc. Lond. B. 266 (1424): 1127-1132.

Davis RW, Fuiman LA, Williams TM, Collier SO, Hagey WP, Kanatous SB, Kohin S, Horning M (1999) Hunting Behavior of a Marine Mammal Beneath the Antarctic Fast Ice. Science 283: 993-996.

Horning, M., Trillmich, F. (1997) Ontogeny of Diving Behaviour in the Galapagos Fur Seal. Behaviour 134: 1211-1257.

Horning, M., Trillmich, F. (1997) Development of Hemoglobin, Hematocrit and Erythrocyte Values in Galápagos Fur Seals. Mar Mam Sci 13(1): 100-113.

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EDUCATION

Ph.D. (Biology) 1995-1999

B.Sc. (Honours Biology) 1990-1994

Dalhousie University, Halifax, Nova Scotia Canada

PROFESSIONAL EXPERIENCE

Assistant Research Professor 2001-present
School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, Alaska

Postdoctoral Fellow 1999-2001
Texas Institute of Oceanography, Texas A&M University at Galveston, Texas

SELECTED PUBLICATIONS

Thomton J, Mellish J (in press) Haptoglobin concentrations in free-range and temporarily captive juvenile Steller sea lions. Journal of Wildlife Diseases.

Mellish J, Horning M, York A (in press) Seasonal and spatial blubber depth changes in captive harbor seals (*Phoca vitulina*) and Steller's sea lions (*Eumetopias jubatus*). Journal of Mammalogy.

Mellish J, Calkins D, Christen D, Horning M, Rea L, Atkinson S (2006) Temporary captivity as a research tool: Comprehensive study of wild pinnipeds under controlled conditions. Aquatic Mammals 32: 58-65.

Mellish J, Iverson S (2005) Postpartum dynamics of reproductive hormones in grey and hooded seals. Marine Mammal Science 21: 162-168.

Mellish J, Tuomi P, Horning M (2004) Assessment of ultrasound imaging as a non-invasive measure of blubber thickness in pinnipeds. Journal of Zoo and Wildlife Medicine 35: 116-118.

Mellish J, Loughlin T (2003) Lipoprotein lipase in lactating and neonatal northern fur seals: exploring physiological management of energetic conflicts. Comparative Biochemistry and Physiology Part A 134: 147-156.

Mellish J, Iverson S (2001) Blood metabolites as indicators of nutrients utilization in fasting, lactating phocid seals: does depletion of nutrient reserves terminate lactation? Canadian Journal of Zoology 79: 303-311.

Mellish J, Iverson S, Bowen D (2000) Metabolic compensation during high energy output in fasting, lactating grey seals (*Halichoerus grypus*): metabolic ceilings revisited. Proceedings of the Royal Society of London, Biological Sciences 267: 1245-1251.

Mellish J, Iverson S, Bowen D (1999) Variation in milk production and lactation performance in grey seals and consequences for pup growth and weaning characteristics. Physiological and Biochemical Zoology 72:677-690.

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EDUCATION

1993 - Doctor of Veterinary Medicine, University of Guelph
1999 - Master of Science (Pathobiology), University of Guelph

EMPLOYMENT

1997 to present - Staff Veterinarian, The Marine Mammal Center, Sausalito, CA
1995 to 1996 - Veterinary Intern, Mystic Aquarium, Mystic, CT
1993 to 1995 - Associate Veterinarian, The Links Road Animal Clinic, Willowdale, Ont.

PUBLICATIONS

Haulena, M. and F.M.D. Gulland. 2001. Use of medetomidine-zolazepam-tiletamine with and without atipamezole reversal to immobilize captive California sea lions. Journal of Wildlife Diseases 37: 566-573.

Haulena, M. and R.B. Heath. 2001. Marine mammal anesthesia. *In*: CRC Hanbook of Marine Mammal Medicine, Second Edition, L.A. Dierauf and F.M.D. Gulland (eds.), CRC Press LLC, Boca Raton. Pp. 655-688.

Haulena, M., F.M.D. Gulland, D.G. Calkins and T.R. Spraker. 2000. Immobilization of California sea lions (*Zalophus californianus*) using medetomidine plus ketamine, alone and in combination with isoflurane, and reversal with atipamezole. Journal of Wildlife Diseases 36: 124-130.

Haulena, M., D.J. St. Aubin and P.J. Duignan. 1998. Thyroid hormone dynamics during the nursing period in harbour seals, *Phoca vitulina*. Canadian Journal of Zoology 76: 48-55.

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Gulland, F.M.D., **M. Haulena** and L.A. Dierauf. 2001. Seal and sea lion medicine. *In*: CRC Handbook of Marine Mammal Medicine, Second Edition, L.A. Dierauf and F.M.D. Gulland (eds.), CRC Press LLC, Boca Raton. Pp.

Gulland, F.M.D., **M. Haulena**, S. Elliott, S. Thornton and L. Gage. 1999. Anesthesia of juvenile Pacific harbor seals using propofol alone and in combination with isoflurane. Marine Mammal Science: 15: 234-235.

Gulland, F.M.D., **M. Haulena**, L.J. Lowenstine, C. Munro, P.A. Graham, J. Bauman and J. Harvey. 1999. Adrenal function in wild and rehabilitated Pacific harbor seals (*Phoca vitulina richardsii*), and in seals with phocine herpesvirus-associated adrenal necrosis. Marine Mammal Science 15: 810-827.

Ostland, V.E., P.J. Byrne, J.H. Lumsden, D.D. MacPhee, J.D. Derksen, **M. Haulena**, G.A. Skar, E. Myhr and H.W. Ferguson. 1999. Atypical bacterial gill disease: a new form of bacterial gill disease affecting intensively reared salmonids. Journal of Fish Diseases 22: 351-358.

King, D.P., A.R. Lie, T. Goldstein, B.M. Aldridge, F.M.D. Gulland, **M. Haulena**, M.A. Adkinson, L.J. Lowenstine and J.L. Stott. 2001. Humoral immune responses to phocine herpesvirus-1 in Pacific harbor seals (*Phoca vitulina richardsii*) during and outbreak of clinical disease. Veterinary Microbiology 80: 1-8.

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Education

Doctor of Veterinary Medicine (Honours) 1970
Washington State University-Pullman, Washington

Professional Experience

Veterinarian, Alaska SeaLife Center - Seward, Alaska 1997- present

Practicing Veterinarian, College Village Animal Clinic 1971- present
Anchorage, Alaska

Veterinary Consultant 1995 - present
- U.S. Fish and Wildlife Service
  - Sea Otter Abdominal Transmitter Implants - Cordova, Alaska
  - Walrus Research Project - Cape Peirce, Alaska
  - Common Murre Transmitter Implants - Homer, Alaska
  - Brandt Geese Heart Rate Transmitter Implants- Anchorage, Alaska
  - Sea Otter Pup Nursery Care and International Transport
- National Marine Fisheries Service
  - Juvenile Steller Sea Lion Field Anesthesia
- Canadian Wildlife Service, North Slope Borough, US Fish and Wildlife Service
  - King, Spectacled and Common Eider Satellite Transmitter Implants

Professional Associations/Honors

American Veterinary Medical Association	1970 - present
Alaska State Veterinary Medical Association	1970 - present
President	1974 - 1975
Chairman - Peer Review Committee	1988 - 1995
American Animal Hospital Association	1974 - present
Regional Practitioner of the Year	1989
Association of Avian Veterinarians	1982 - present
State Member Representative	1992 - present
International Association of Aquatic Animal Medicine	1990 - present
American Association of Wildlife Veterinarians	1997 - present
American Association of Zoo Veterinarians	1998 - present

Selected Publications

Tuomi, P., 2001, "Sea Otters" in: Handbook of Marine Mammal Medicine, Dierauf, L.A. and F.M.D. Gulland, eds., CRC Press, Baca Raton, FL pg. 961-988.

Tuomi, P., M. Grey, and D. Christen, 2000, "Butorphanol and Butorphanol/ Diazepam Administration for Analgesia and Sedation of Harbor Seals *(Phoca vitulin*a)", in: Proceedings of American Association of Zoo Veterinarians and International. Association of Aquatic Animal Medicine Joint Conference, Baer, C.K. and R.A. Patterson, eds., New Orleans, LA, pg 382-383.

Tuomi, P., and T. Williams, 1995, "Effects of Oiling and Rehabilitation on Pregnant and Newborn Sea Otters", in: Proceedings of IWRC Oiled Wildlife Symposium, Seattle, Wa., Rineer-Garber, C., ed., pg.218-221.

Tuomi, P., D.M. Mulcahy and G.W Garner, 1996, "Immobilization of Pacific Walrus *(Odobenus rosmarus divergens)* with carfentinil Reversal and isoflurane anesthesia" in: Proceedings of International Association for Aquatic Animal Medicine, Abt, D.A., ed., Chattanooga, TN., Vol. 27, pg. 121-123.

Tuomi, P., S. Donoghue, J. Otten-Stranger, 1995. "Husbandry and Nutrition", in: Emergency Care and Rehabilitation of Oiled Sea Otters: A Guide for Oil Spills Involving Fur-bearing Marine Mammals, Williams, T.M. and R.W.Davis, eds., University of Ak. Press, Fairbanks, Ak., pg. 103-120.

Williams, T., R. Davis, J. McBain, **P. Tuom**i, R. Wilson, C. McCormick, and S. Donoghue, 1995. "Diagnosing and Treating Common Clinical Disorders In Oiled Sea Otters". Ibid, pg. 59-94.

Tuomi, P., 1990, "Husbandry - Valdez Rehabilitation Center", in: Bayha, K. and J. Kormendy, Tech. coord., Proceedings of the Sea Otter Symposium Following the T/V Exxon Valdez Oil Spill, Anchorage, Alaska, April 17-19, 1990, USFWS Biol. Rep. 90(12), pg. 274-284.

Harris, R.K., Moeller, R.B., Lipscomb, T.P., Haebler, R.J., **Tuomi, P.**A., McCormick, C.A., DeGange, A.R., Mulcahey, D., Williams, T.D., and Pletcher, J.M., 1990, "Identification of a herpes-like virus in sea otters during rehabilitation after the T/V Exxon Valdez oil spill." In: Sea Otter Symposium: Proceedings of a symposium to evaluate the response effort on behalf of sea otters after the T/V Exxon Valdez oil spill into Prince William Sound, Anchorage, AK 17-19 April 1990. K. Bayha and J. Kormendy, (eds), U.S. Fish and Wildlife Service Biological Report, 90,12: 366-368.

Tuomi, P., 1990, "Rehabilitation Notes: Sea Otter Pups *(Enhydra lutri*s)", Wildlife Journal, International Wildlife Council, Vol. 13, No. 9, pg 9-14.

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EDUCATION

Ph.D. Wildlife Conservation - University of Minnesota, 2001
M. S. Wildlife Conservation - University of Minnesota, 1996
B. S. Fisheries and Wildlife Management - Montana State University, 1987

EMPLOYMENT

Sept, 2004 - Present, Leader, Alaska Ecosystem Program, National Marine Mammal Lab, NMFS, NOAA GS-14

June, 2003 – Sept, 2004, Principal Investigator, Steller Sea Lion Program, Alaska Dept. of Fish and Game, WBIV Series 20D

March, 2001 – June 2003, Principal Investigator, Steller Sea Lion Program, Alaska Dept. of Fish and Game, WBIII Series 18C

1992 - 2001, Graduate Research Assistant, University of Minnesota

1991 - 1996, District Wildlife Biologist, Targhee National Forest, Ashton, Idaho, GS-486-9 (On leave 1992 - 1996)

1989 - 1991, Assistant District Wildlife Biologist, Targhee National Forest, Island Park, Idaho, GS-486-7

1987 - 1989, Wildlife Technician, Targhee National Forest, Island Park, Idaho GS-404-5

1986, Biological Aid, Idaho Dept. Fish and Game, Lewiston, ID, June ~ GS-4

PUBLICATIONS

Buckles, E. L., B. M. Aldridge, T. S. Gelatt, P. Ross, M. Haulena, L. J. Lowenstine, and F. M. D. Gulland. 2004. Fetus in fetu in a harbor seal (*Phoca vitulina Richardsi*): histopathological genetic and toxicological analysis. *In Review*, Journal of Wildlife Diseases.

Burek, K. A., K. Beckmen, T. Gelatt, W. Fraser, A. J. Bracht, K. A. Smolarek, and C. H. Romero. . Poxvirus infection of Steller sea lions (*Eumetopias Jubatus*) in Alaska. *Accepted*, Journal of Wildlife Diseases.

Raum-Suryan, K. L., M. J. Rehberg, G. W. Pendleton, K. W. Pitcher, and T. S. Gelatt. 2004. Dispersal, movement patterns, and haulout use of pup and juvenile Steller sea lions (*Eumetopias jubatus*) in Alaska. *In press*, Marine Mammal Science.

Stewart B. S., P. K. Yochem, T. S. Gelatt & D. B. Siniff. 2003. The pack ice niche of Weddell seals in the Ross Sea. Pp. 224-228, In: AHL Huiskes et al. (eds) Antarctic Biology in a Global Context (Backhuys Publ, Leiden, The Netherlands.

Davis, C. S., T. Gelatt, D. Siniff, I. Stirling, and C. Strobeck. 2002. Dinucleotide microsatellite markers from the Antarctic Monachines and their use in other pinnipeds. Molecular Ecology Notes 2:203-208.

Gelatt, T. S., D. B. Siniff, and J. A. Estes. 2002. Activity patterns and time budgets of sea otters during the population decline at Amchitka Island, Alaska. Journal of Wildlife Management 66: 29-39.

Gelatt, T. S., C. S. Davis, D. B. Siniff, and C. Strobeck. 2001. Molecular evidence for twinning in Weddell seals. Journal of Mammalogy 82:491-499.

Gelatt, T., C. Davis, M. Cameron, D. Siniff, and C. Strobeck. 2000. The old and the new: integrating population ecology and population genetics of Weddell seals. Pages 63-70 *in*: Antarctic ecosystems: models for wider ecological understanding, *eds* Davison, W., C. Howard-Williams, and P. Broady. New Zealand Natural Sciences. 332 pp.

Stewart, B. S., P. K. Yochem, T. S. Gelatt, and D. B. Siniff. 2000. Dispersion and habitat use of Weddell seals (*Leptonychotes weddellii*) in the Ross Sea, Antarctica, during their first year of life. *in*: Antarctic ecosystems: models for wider ecological understanding, *eds* Davison, W., C. Howard-Williams, and P. Broady. New Zealand Natural Sciences. 332 pp.

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Gelatt, T. S., and D. B. Siniff. 1999. Line transect survey of Antarctic seals in the Amundsen-Bellingshausen Seas, 1994. Wildlife Society Bulletin. 27:330-336.

Gelatt, T. S., and D. B. Siniff. 1998. Population ecology of Weddell seals 1995-1997. Antarctic Journal Review 1997: 108-109.

Gelatt, T. S. and J. D. Kelley. 1996. Western painted turtles *Chrysemys picta belli* basking on a nesting common loon *Gavia immer*. Canadian Field-Naturalist. 109:456-458.

Gelatt, T. S., S. Hill, and B. D. Scotten. 1994. Research to investigate pack-ice seal activities during Nathaniel B. Palmer cruise 94-2. Antarctic Journal Review 1994:120-121.

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Education:

1995 Doctor of Philosophy, University of Alaska, Fairbanks, Alaska
Dissertation title: Prolonged fasting in Pinnipeds. Chair: Dr. Michael A. Castellini

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Thesis title: Changes in resting metabolic rate during long-term fasting in northern elephant seal pups (Mirounga angustirostris). Chair: Dr. Daniel P. Costa

1987 Bachelor of Science (Honors Marine Biology), University of Guelph, Guelph, Ontario, Canada.

Professional Affiliations:

Wildlife Biologist IV, Marine Mammals Section, Division of Wildlife Conservation, Alaska Department of Fish and Game, Fairbanks, AK 99775 (August 2004 to present)

Affiliate Assistant Professor, Environmental and Natural Resources Institute, University of Alaska, Anchorage, AK 99501 (November 2001 – present).

Affiliate Assistant Professor of Marine Science, University of Alaska, Fairbanks, Alaska 99775 (October 2000 – June 2005)

Affiliate Assistant Professor, Department of Biology, University of Central Florida, Orlando, FL (August 2001- present)

Past Professional Affiliations:

Wildlife Biologist III, Marine Mammals Section, Division of Wildlife Conservation, Alaska Department of Fish and Game, Anchorage, AK 99518 (August 2000 to August 2004)

Assistant Professor, Department of Biology, University of Central Florida, Orlando, FL (1998 to 2000, leave of absence status August 2000 to August 2001)

Research Associate, Institute of Marine Science, University of Alaska, Fairbanks, AK (May-June 1998)

Research Biologist, National Marine Mammal Laboratory, NMFS, Seattle, WA (April-May 1998)

Research Associate, National Research Council, tenured at the National Marine Mammal Lab, National Marine Fisheries Service, NOAA, Seattle, WA (1995 to 1997)

Graduate Research Assistant, Institute of Marine Science, University of Alaska (1990 to 1995)

Graduate Teaching and Research Assistant, Institute of Marine Sciences, University of California, Santa Cruz, CA (1987 to 1990)

Assistant Research Biologist, Tarandus Associates, Toronto, Canada (1987, seasonal)

Assistant Research Biologist, Ontario Hydro Corp., Biological Research Section, Toronto, Canada (1985 and 1986, seasonal)

Selected Publications:

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APPENDIX 2

Table 2. Implanted telemetry papers by category.

General Technology	Mammals	Aquatic Mammals	Species
Folk et al. 1971, Neely & Campbell 1973, Smith & Whitney 1977, MacDonald & Amlaner 1980			
	Koehler et al. 1987, Rawson & Hartline 1964.		Ord's Kangaroo rat, Montane vole, Deer mouse, Townsend's ground squirrel
	Van Vuren 1989		Yellow-bellied marmot
	Agren et al. 2000.		European badger
	Madison 1980		Meadow vole
	Eagle et al. 1984.		American mink, Franklin's ground squirrel
	Smith 1980 a,b.		White-footed mice
	Philo & Follman 1981		Grizzly bear
	Moe et al. 1995, Bakken et al. 1999		Silver fox
		Ranheim et al. 2004, Wheatley 1997, Guynn et al. 1987, Davis et al. 1984	Beaver
		Ralls & Siniff 1990, Ralls et al 1989, Siniff 1985, Siniff & Ralls 1991, Monnett & Rotterman 2000, Garshelis & Siniff 1983, Williams & Siniff 1983	Sea otter
		Hernandez-Divers et al. 2001, Johnson & Berkley 1999, Reid et al. 1986, Hoover 1984, Melquist et al. 1981, Melquist & Hornocker 1979,	River otter
		Mulcahy & Garner 1999	Polar bear

Appendix 3.
Pre-LHX implantation juvenile Steller sea lion Health Screening Protocol.

All animals will undergo a health screen protocol prior to implantation of LHX tags. Screening will consist of physical inspection of broken bones, major wounds and/or inflammation, assessment of body condition via ultrasound, and collection and analysis of samples. Sample collection includes: blood, swabs, feces, mucus, blubber, skin and hair. All animals are sampled at capture (both juveniles that will receive LHX implants and those that are immediately released). Sample-based health screening will consist of the immediate, on-site examination of whole blood for hematologic analysis (WBC, Differential count, RBC, HCT, HgB, MCV, MCH, MCHC, Platelets) and harvesting of serum for immediate on-site serum biochemistry analysis (Glucose, BUN**,** Creatinine, Potassium Calcium, Albumin, Globulin, Total protein, Total bilirubin, Alkaline phosphatase, ALT, Amylase, Cholesterol). On site analysis will be done via portable haematology and clinical chemistry analyzer (Vetscan / HMT or equivalent).
No specific thresholds will be set for any individual sampled / assayed parameter. Instead, attending veterinarian(s) will make an on-site comprehensive differential health assessment using standard veterinary methods to deem an animal healthy and suitable for implantation surgery.
Subsequently, serologic analysis will be performed to test for evidence of exposure to common marine mammal and terrestrial mammal pathogens. Sera are submitted for testing for antibodies to bacterial pathogens: *Brucella spp.*, *Leptospira spp.* (serovars *bratislava, canicola, grippophytosa**,** hardjo, icterohaemorrhagica, pomona)*; viruses: Morbilliviruses (canine distemper, phocine distem per, dolphin morbillivirus, porpoise morbillivirus), Phocine herpesvirus-1, Calicivirus serotype 1(San Miguel sea lion virus); and protozoa: *Toxoplasma gondii*, *Sarcocystis neurona*.

Microbiologic analysis is performed to identify normal and pathogenic flora. Sampling includes collection of nasal, vaginal (females) and preputial (males) swabs into Amies gel media and rectal swabs into Cary Blair gel media. Obtained *E. coli* isolates from rectal swabs are tested for antibiotic resistance to 12 commonly used antibiotics (Amikacin, Amoxicillin/clavulanic acid, Ampicillin, Cefazolin, Ceftiofur, Ceftizoxime, Chloramphenicol, Enrofloxacin, Gentamicin, Tetracycline, Ticarcillin/clavulanic acid, Trimethoprim sulfur)

Identification of endo and ecto parasites is performed to monitor parasite loads over time. Sampling includes collection of tracheal mucous following extubation and feces.

Appendix 4 - Emergency Drug List

DRUG	20 - 40 kg	60 -105 kg	200-250kg	750 - 900 kg	950 - 1100 kg	ROUTE	USE	COMMENT
**Aminophylline (25mg/ml) Dose: 5.5mg/kg	4.4 - 8.8 ml	13.2 - 23 ml	44 - 55 ml	165-198 ml	209 - 242 ml	IV, IM, PO	Bronchodilation	Compatible with Dexamethasone and lidocaine.
Atropine (0.54mg/ml) SA Dose: 0.02mg/kg	0.74 - 1.48 ml	2.2 - 4.2 ml	7.4 - 9.2 ml			IM or IV	Bradycardia	May increase the effects of epinephrine.
Atropine (15mg/ml) LA Dose: 0.02mg/kg			0.26 - 0.33 ml	1 - 1.2 ml	1.2 - 1.4 ml	IM or IV	Bradycardia	LARGE ANIMAL CONCENTRATION
**Dexamethasone (4mg/ml)								Compatible
Dose: 0.2 - 1mg/kg	1 - 2 ml	3 - 5.2 ml	10 - 12.5 ml	37.5 - 45 ml	47.5 - 55 ml	IM, PO	Anti - inflammatory	with aminophylline
Dose: 2.2mg/kg	11 - 22 ml	33 - 57 ml	50 - 100 ml	Max 100 ml	Max 100 ml	IV	Shock	and lidocaine.
Diazepam (5mg/ml) Dose: 1 - 5 mg/kg	4 - 8 ml	12 - 21 ml	40 - 50ml	150- 180 ml	190 - 220 ml	IV, IM	Seizure control	
Dopram-V (20mg/ml) Dose: 5 - 10mg/kg	3 - 5 ml	5 - 10 ml	20 - 30 ml	Start w/ 20ml Max.100 ml	Start w/ 20ml Max.100 ml	IV, Sublingual, IM (use last), or partial IT	Post-anesthetic CNS/respiratory stimulant	May interact with epinephrine and aminophylline
Epinephrine (1:1000) 1mg/ml Dose: 0.2-0.5 mg/kg	4 - 8 ml	12 - 21 ml	40 - 50 ml	150 - 180 ml	190 - 220 ml	IT, IV, IM, SQ, IC	Anaphylaxis and cardiac resuscitation	Do not give with aminophylline or lidocaine
**Furosemide (50mg/ml) Dose: 2.5 - 5.0 mg/kg	1 - 2 ml	3 - 5.25 ml	10 - 12.5 ml	37.5 - 45 ml	47.5 - 55 ml	IM, PO	Ventricular arrhythmia and tachycardia.	BID
Lidocaine (20mg/ml) Dose: 1 - 2mg/kg	1 - 2 ml	3 - 5.25 ml	10 - 12.5 ml	37.5 - 45 ml	47.5 - 55 ml	IV SLOWLY		Do not give with epinephrine. Compatible with aminophylline and Dexamethasone.

Sources: **CRC Handbook of Marine Mammal Medicine; Marine Mammal Center, Drug Formulary; ASLC Drug Formulary

APPENDIX 5

Contingency plan for complications that may arise prior to release of an animal

This is a contingency plan that describes how we may proceed in the event of complications encountered during or after LHX tag implant surgeries. It is important to point out that we do not anticipate the need to apply the contingency plan. However, when performing surgery under field conditions unpredictable situations may arise. The purpose of this contingency plan is to discuss possible complications, and possible scenarios and remedial approaches for dealing with such complications. At the same time, a key element of a contingency plan is the need to call this plan into action in the case of unplanned and to some extent unpredictable events. Therefore, retaining the ability to conduct a flexible response to complications is essential. As a result, this contingency plan outlines a list of possible complications, and a list of possible actions that could be taken in response to some of these complications. Neither list is in any way exhaustive, and the purpose of the contingency plan is not to detail a rigid and specific course of action to be taken in response to specific complications. Instead, the purpose of this plan is to demonstrate that to the maximum possible extent, precautions and preparations will be taken to ensure well being and safety of animals and project participants. In addition, the purpose of this plan is to ensure that the Principal Investigator and/or co-Investigators, under the guidance of attending veterinarians, can legally conduct such unanticipated remedial actions the suitability of which can only be determined on site by qualified personnel based on the situation and state of the animal.

Non-exhaustive list of possible complications that may arise prior to release of an animal:

1) Peritonitis
2) Surgical infection
3) Wound herniation / dehiscence
4) Gastro-intestinal paralysis from handling (ileus)
5) Pain, fear
6) Underlying metabolic problem that may be related to recent anesthesia and/or surgery, including:
   infection, cardiac or cardiovascular insufficiency, respiratory insufficiency, neurological disorder, renal or hepatic insufficiency, and others.
7) Underlying metabolic problem, infection or injury that may be unrelated to recent anesthesia and/or surgery, but was not diagnosed prior to surgery, or was diagnosed during initial anesthesia (in which case surgery would not be conducted), including:
   problems listed under 6, and parasites, immune system deficiencies, broken bones, myositis, and others.

Non-exhaustive list of assessment procedures that may be conducted for the purpose of determining the state of the animal, and the nature of the complication (if required equipment available, these procedures can be conducted ship-board):

1) Visual observation

2) Infrared thermography or non-contact temperature measurement
3) Restraint and/or immobilization, tranquilization, anesthesia
4) Physical exam with visual inspection, palpation, rectal temperature collection
5) Ultrasound
6) Portable x-ray
7) Blood sampling for CBC with differential and blood chemistry
8) Endoscopy or laprascopy
9) Exploratory surgery

Non-exhaustive list of possible remedial actions to be taken in response to complications that may arise prior to the release of an animal:

1) Treatment with drugs (P.O., I.M., or I.V.) including (but not limited to): analgesics or indicated pain medication, sedatives, vitamins, non-steroidal anti-inflammatory drugs (NSAID), gastric motility drugs, broad-spectrum antibiotics, I.V. fluids, as indicated.
2) Extended maintenance of animal onboard the ship in holding cages, for up to 7 days after initial capture. Extended maintenance may be done in multiple steps, with multiple health assessments and physical exams, as required. Physical maintenance of animal may include forced feeding and/or I.V. drips.
3) Surgery, including laprascopic/endoscopic surgery, wound treatment surgery, re-suturing a herniated wound, and tag explant surgery.
4) Euthanasia (as described in ***Appendix 6***). It is important to point out that death as an endpoint is not part of the project plan, but given that surgeries will be conducted in the field, we are required by the IACUC process to include and specify a planned method of euthanasia, and include it's possible application under the guidance of qualified veterinarians, in all permit applications. A euthanasia would count as an unintentional mortality under this permit.

If contraindications no longer exist after appropriate treatments / actions, the animal will be released.

APPENDIX 6

Euthanasia Protocol

Excerpt from:
Alaska SeaLife Center
Euthanasia Protocol

All methods of euthanasia must follow the recommendations of the 2000 Report of the AVMA Panel on Euthanasia (Journal of American Veterinary Medical Association Vol. 218, No. 5, March 15, 2001, pages 669-696).

The standard method of euthanasia for all mammal species will be by intravenous or intraperitoneal injection of Buethanasia (or comparable pentobarbitol based injectable euthanasia agent) at a rate of 2.5 ml per 10 kg (1ml per 10 lb) body weight. Cessation of heart beat and respiration will be assessed to insure death.

When necessary for human safety or to avoid unnecessary distress, animals may first be sedated or immobilized by appropriate inhalant gas anesthesia (isoflurane) or intramuscular injectable agents such as telazol, ketamine, diazepam, or medetomidine at dosage rates noted in the current ASLC formulary for that species. Oral dosing with pentobarbitol may provide fair to adequate degrees of sedation prior to injection but is unreliable and should be used only as a last resort. Potent opiods such as etorphine hydrochloride (M 99) or carfentanyl administered by intramuscular injection via dart or pole syringe may be used for larger mammals but are not currently kept at the ASLC.

Note that all of the injectable agents are controlled substances that must be purchased and administered by a Drug Enforcement Agency licensee. All DEA controlled substances must be held in the locked secure cabinet in the Veterinary Clinic and proper records maintained at all times. Please refer to ASLC Controlled Drug Protocol (below). Carcasses of animals must be properly disposed of by incineration or deep burial at an approved site after administration of any of these agents to prevent accidental ingestion by scavengers or contamination of the environment.

All research protocols shall specify a planned method of euthanasia and carcass disposition for use in case of emergencies even if death as an end point is not part of the proposal. The IACUC Assurance of Animal Care Form for mammals should indicate injection of pentobarbitol euthanasia solution as described above and refer to this protocol as a default plan.

Collection, research or rehabilitation animals may be subject to euthanasia based on the recommendations of the ASLC Veterinarian due to one or more of the following circumstances:

1. Illness or injury causing permanent or untreatable pain or suffering.
2. Inability to swim or otherwise move within its environment in a manner adequate for feeding, social interaction and other normal activities.
3. Contagious or infectious disease which cannot be controlled or cured.
4. Non releasable animals for which adequate long term captive care facilities cannot be located.
5. As directed by permitting agencies.

In emergency situations, every effort will be made to contact the appropriate persons (Curator, Principle Investigator or designee, Husbandry or Rehabilitation Director, etc) to discuss euthanasia recommendations. However, in an emergency, the Veterinarian reserves the option of unilateral action in the case of intractable pain and suffering or uncontrollably dangerous animals if contacts cannot be made in a timely manner.

Documentation shall be completed for any animal where euthanasia is performed and at least one copy will be kept permanently on file. The documentation shall include the identification of the animal, the reason (s) for euthanasia and the date and method of euthanasia and disposal.

1. Collection animals

   Documentation shall be signed by the Husbandry Director , the appropriate Curator, and the veterinarian for planned euthanasia or by the Executive Director or acting director and the veterinarian for emergency euthanasia.

2. Research animals

   Documentation shall be signed by the Principal Investigator or the project on site designee, the Research Director, and the veterinarian for planned euthanasia or by the Research Director or acting director and the veterinarian for emergency euthanasia.

3. Rehabilitation animals

   Documentation shall be signed by the Rehabilitation Manager or their designee and the veterinarian for planned euthanasia or by the Executive Director or acting director and the veterinarian for emergency euthanasia.

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