I. APPLICATION FOR A PERMIT FOR SCIENTIFIC RESEARCH UNDER THE MARINE MAMMAL PROTECTION ACT AND FOR SCIENTIFIC PURPOSES UNDER THE ENDANGERED SPECIES ACT. Current Permit No: 358-1769

II. DATE OF APPLICATION 1 December 2006

III. APPLICANT AND PERSONNEL

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B. Experience and Qualifications

Curriculum Vitas for the Principal Investigator and Co-investigators listed in this application are on file with the office of Protected Resources. Table 1 lists activities to be carried out by each PI and CI. All research, and contracts awarded for research under this permit, will be supervised by the Principal Investigator and/or Co-Investigators.

IV. DESCRIPTION OF PROPOSED SCIENTIFIC RESEARCH

A. Summary

This permit application covers the long-term Steller sea lion *(Eumetopias jubatus)* research program for the Alaska Department of Fish and Game (ADFG). The research program is charged with investigating the various hypotheses for the decline or lack of recovery of Steller sea lions in Alaska. As such, the permit covers a wide variety of research activities and many different kinds of takes, including incidental disturbance during aerial surveys (up to 20,000 individuals in the eastern DPS disturbed per year), disturbance of animals on rookeries and haulouts during brand resighting surveys (up to 25,000 individuals in the eastern DPS and up to 5,000 individuals in the western DPS disturbed per year) and incidental to scat collection, capture for instrument attachment, physiological research and sample collection (up to15,000 individuals in the eastern DPS and 2,000 in the western DPS disturbed per year), permanent marking of pups for long-term demographic and distribution studies (up to 800 marked per year), capture of up to 280 older animals for physiological assessment and attachment of scientific instruments to investigate foraging ecology and diving behavior (up to 95 instrumented per year). Harbor seals (*Phoca vitulina richardsi*), northern fur seals (*Callorhinus ursinus*), and California sea lions (*Zalophus californianus*) may be disturbed incidentally during the course of this research due to proximity of isolated individuals to the Steller sea lion study area. Field and laboratory work will take place during all seasons of the year and throughout the range of the Steller sea lions in Alaska (both eastern and western DPS). This permit does not involve intentional lethal take or captive removal of marine mammals. The requested period of this permit is June 1, 2007 to May 31, 2012.

B. Introduction

1. Species and Status of Affected Stocks:

We request authority to take Steller sea lions (*Eumetopias jubatus*) during 5 years of continuing research, through 31 May 2012. This request includes animals from both the eastern and western stocks (east and west of 144° W longitude, respectively) throughout its range of distribution in Alaska. We also request authority for inadvertent disturbance of harbor seals (*Phoca vitulina richardsi*) of the Bering Sea, Gulf of Alaska, and Southeast Alaska stocks and northern fur seals (*Callorhinus ursinus*) at Hazy Island, Southeast Alaska during Steller sea lion research activities in Alaska.

Steller sea lions were listed as “threatened” range-wide under the U.S. Endangered Species Act (ESA) on 26 November 1990 (55 Federal Register 49204). The population includes two stocks (eastern and western), separated at 144° W longitude (Loughlin 1997). The western stock was listed as “endangered” under the ESA on 4 May 1997 and the eastern stock remains classified as “threatened”

(62 FR 24345). Steller sea lions are listed as "depleted" under the MMPA. Both stocks are thus classified as strategic. Detailed reviews of Steller sea lion population status can be found in biological consultations for groundfish fisheries (NMFS 2001), supplemental environmental impact statements (NMFS 2002), and by the National Research Council (NRC 2003). Annual updates of stock status are also presented in the Alaska Marine Mammal Stock Assessment Reports (Angliss and Lodge 2004).

Northern fur seals (Pribilof Islands and Eastern Pacific) are classified as a strategic stock and listed as "depleted" under the MMPA.

Harbor seal stocks (Bering Sea, Gulf of Alaska, Southeast Alaska, Washington, Oregon, and California stocks) are not classified as strategic stocks, nor are they listed as "depleted" under the MMPA, or as "threatened" or "endangered" under the ESA.

2. Background/Literature Review

This permit application covers the long-term Steller sea lion research program for the Alaska Department of Fish and Game (ADFG) that has been conducted under MMPA and ESA Permit #358-1564 and its amendments and #358-1769. As such, the permit covers a wide variety of research activities and many different kinds of takes, including incidental disturbance during aerial surveys (NMFS 1992 research task 321 and 341: NMFS 2006 task 1.1), disturbance of animals on rookeries and haulouts during resighting surveys (task 1.2.1), scat collection (research task 6 and 614), and capture activities (task 1.4.1), instrument attachment (task 2.3.3), physiological research and sample collection (task 1.3.2), marking of pups for long-term demographic and distribution studies (task 1.2.1), and attachment of scientific instruments to investigate foraging ecology and diving behavior (task 2.5.1). These activities address the most pressing research needs as identified by the Steller Sea Lion Recovery Team (National Marine Fisheries Service 1992), incorporating revisions and advice that ensued from workshops convened by the Recovery Team during 1998 and 1999 to review telemetry, physiology, and behavior/rookery-based research. Additional direction has also come from workshops convened to address the leading hypotheses addressing the decline of Steller sea lions in the Western Stock, and a National Research Council Review of current research, as well as the draft 2006 Recovery Plan. Field and laboratory work will take place during all seasons of the year and throughout the range of the Steller sea lions in Alaska.

During 1997-1999, the Steller Sea Lion Recovery Team conducted peer-reviewed workshops to review research conducted to date in pursuit of the Recovery Plan, and to identify necessary changes in research program emphasis in four subject areas: land-based observational studies of behavior; telemetry studies; physiological studies; and feeding ecology studies (NMFS 1997a,b; 1999a,b). Recommendations for further research studies made by these review panels included: juvenile survival studies, in particular comparing declining versus stable areas; an extensive branding program (with evaluation of costs of associated disturbance and mortality) combined with satellite or other telemetry studies; more telemetry of weaned pups and yearlings; studies to determine lactation physiology and nursing behavior; and to make every effort to evaluate Steller sea lion body condition along their range and at different times in their life history and seasons.

The Recovery Plan (and new draft 2006 Recovery Plan) acknowledges that certain types of research activities, including capturing animals for attaching telemetry instruments or obtaining blood samples, and branding sea lions are intrusive and cause disturbance and could lead to a lethal take. Despite this intrusiveness, the Plan recommends including such studies in conjunction with other activities, evaluating the potential benefits on a case-by-case basis using the best current information at the time of the permit application. The Plan encourages use of mitigation measures to minimize the impacts of research and recommends development of alternative, less intrusive, techniques for collecting data. Additionally, the need to handle animals in poor and dying condition to assess potential factors affecting the health of the population recognizes that on occasion those animals may succumb, while under the control of researchers, for reasons unrelated to their capture. However, to avoid handling such animals for fear of mortality is to ignore what could potentially be one of the most valuable and informative samples available.

In a review of fishery management actions proposed in the NMFS (2000) groundfish biological opinion by the Alaska Steller Sea Lion Restoration Team, the Team also presented research recommendations designed to obtain a greater understanding of sea lion biological and ecological processes, and to determine the probability of competition for food between sea lions and fisheries (Kruse et al., 2001). The team recommended that particular research emphasis be placed on adult females and subadults of both sexes, especially pre-weaning and during the first 1-2 years post-weaning. Among other recommendations, they specifically recommended studies to: estimate current age-specific survival and reproduction rates; explore the potential impact of disease and contaminants on sea lion populations; continue genetic analyses to further determine population structure, determine the seasonal (breeding vs. non-breeding) use patterns of rookeries and haulouts; continue to develop additional indices of nutritional status; determine that sea lion body condition responds to changes in food availability (quantity and quality), and subsequent relationships with vital population parameters; and to develop techniques to identify when weaning occurs. In a separate review of this Opinion prepared for the North Pacific Fishery Management Council, Bowen et al. (2001) recommended priority be given to research on monitoring trends in population size and distribution, estimation of vital rates, understanding spatial and temporal scales of foraging, and estimating diet.

NRC (2003) reviewed available data in evaluating multiple hypotheses for the population decline, and provided recommendations for continued research and monitoring. They recommended directing research toward measuring vital rates and response variables most indicative of Steller sea lion population status. This included monitoring population trends utilizing aerial surveys of juveniles and adults combined with direct pup counts at rookeries; determination of vital rates (fecundity, age at first reproduction, age distribution, juvenile survival, adult survival, growth rates) through increased branding and resighting efforts; and document at-sea distribution and foraging activity through use of telemetry devices including stomach telemetry tags.

As in the past, the research detailed in this application has been designed to address some of these topics. This includes the continuation of long-term animal marking and resighting programs and the continued modification of capture and handling techniques. The entire pup branding and resighting work is intended to provide basic life history data necessary as a baseline in virtually any other population ecology or physiological study. Although the activities of this program are similar to previous years, this data must be collected on an annual basis to determine changes in population dynamics or health and condition of animals between years, stocks, and seasons.

3. Hypothesis/Objectives and Justification

The purpose of this research is to continue monitoring the status of the Alaskan Steller sea lion population and, by monitoring the health, nutritional status and foraging behavior of these animals, investigate the potential causes of the population decline (or lack of recovery) so as to provide for the recovery of the population. By permanently marking (hot branding) these individuals, researchers will be able to identify individual animals, which will aid in long-term studies on the survival rates of Steller sea lions. The three overriding hypotheses to be addressed with this research program, often through comparison with parallel research being conducted in the western stock by National Marine Mammal Laboratory are:

H_{01}: There are no significant differences between vital rates parameters measured in the eastern and western DPS segments (nor changes over time);

H_{02}: There are no significant differences between health and condition indices of eastern and western stock Steller sea lions (nor changes over time); and

H_{03}: There are no significant differences between diet and timing of weaning in eastern and western stock juvenile Steller sea lions (nor changes over time).

Specific objectives of this project to address these hypotheses include:

1) Assess distribution, dispersal, and vital rates such as age-specific survival rates, reproductive rates, and probability of weaning in Steller sea lions, primarily in the eastern DPS.

2) Assess the physical health of free-ranging juvenile Steller sea lions through standard biochemical assays on blood serum, by monitoring tissue samples (blubber, blood, hair and feces) for toxicology to determine if these individuals are contaminated by toxins in their environment, and by monitoring the presence of specific bacteria or viruses in captured Steller sea lions through fecal loops and culture swaps.

3) Monitoring the foraging and diving behavior of individual juvenile animals. This part of the study is conducted through the use of satellite linked location monitors and archival dive recording tags. These instruments will be affixed to the pelage of a subset of individuals and will provide information regarding the geographic location and diving behavior of the animal.

4) Assessing the body condition and energy status of juvenile animals using deuterium oxide dilution methods. Bioelectrical Impedance Analysis (BIA) will also be conducted to test this alternative method of measuring body composition

5) Determining whether individual juveniles are foraging independently on solid prey or consuming milk provided by their mother. This objective will be met through the use of stable isotopes (levels measured in the whisker) and by examining the fatty acid composition of the individuals blubber layer.

6) Investigate the oxygen carrying capacity of individual animals. This objective will be met through the examination of blood and muscle oxygen stores. This component of the study will investigate the

development of oxygen storage capacity and attempt to determine if young animals are limited in their foraging ability due to physiological development.

7) Determine the whole animal metabolic rate of a subset of the juvenile sea lions captured during our physiology research by indirect calorimetry using open circuit respirometry (a portable metabolic chamber).

8) Determining the age of individual juvenile animals through examination of tooth dentin and cementum layers. In order to assess the age of Steller sea lions captured it is necessary at times to take a tooth from the animal. Accurately determining age of captured individuals is required for investigation of age at weaning and growth rates of individuals.

The purpose of this research is to monitor and investigate the vital rates, health, condition and feeding status of free ranging juvenile Steller sea lions. It is not possible to retrieve this information from cell cultures, computer models or cadavers. This study will be comparing the condition and nutritional status of free-ranging juvenile Steller sea lions from various geographic areas (Southeast Alaska, Prince William Sound, Kodiak and Aleutian Islands). Additionally this project aims to examine how the condition of the animals changes with age to identify life history stages that may be at risk of undernutrition if food is a limiting factor to population growth. Inter-annual differences are monitored to detect change in condition of animals that may be related to changes in food availability in the environment. Comparisons between eastern and western stock sea lions provide an opportunity to investigate differences in vital rates, health, condition and diet that may be driving differences in population trajectories in the two DPS. Comparisons to surrogate species such as California sea lions are of limited utility due to habitat and diet difference. The results of these studies are being solicited for use in computer modeling of energetics and population dynamics and to provide the best available information for preparation of Biological opinions and ESA consultations. This research represents continued implementation of the Final Recovery Plan for Steller Sea Lions, specifically (1) the need to identify habitat requirements and protect areas of special biological significance; (2) identify management stocks, (3) monitor status and trends of sea lions; (4) monitor health, condition, and vital parameters; and (5) investigate feeding ecology and factors affecting energetic status. A draft Steller Sea Lion Recovery Plan is currently in review and until it is finalized, guidance for research pursuant to Steller sea lion conservation is available from recommendations made by the Recovery Team (NMFS 1997 a,b and 1999a,b) and the NRC (2003). The types of data required for conservation of Steller sea lions and for evaluation of fisheries management actions must be taken from wild populations of Steller sea lions.

Sample sizes of animals targeted for physiology related work were chosen based on a minimum of 20 animals per 3 month age category/bin within each study area with an effort made to distribute these captures over at least 2 years to address inter-annual variability. Unfortunately, it is not physically possible for us to cover all 4 areas in all seasons each year. Comparisons will be made based upon age-matched samples from areas of low vs. high population decline. Southeast Alaska has been selected as a proxy for a control population given stable population trends compared to other areas, and that pre-decline data of this type is not available for comparison. Given the variability in blood chemistry parameters previously measured, this level of sampling appears to give sufficient statistical power to identify significant differences between areas of study. All animals handled for physiology research are branded for future identification and the potential for longitudinal sampling to monitor changes in physiological parameters with age in one individual. However, these animals are not included in the survivorship modeling since they are not marked as young pups. All research efforts are coordinated closely with researchers at the National Marine Mammal Laboratory, NMFS, with sample sharing to effectively increase sample sizes and geographical coverage of research where possible.

Sample sizes chosen for population dynamics research (branding of young pups) were based on modeling exercises conducted by ADFG and NMFS biometricians in the 1999 branding workshop to determine appropriate sample sizes for this program of study (see also Appendix 1 for complete discussion of branding operations). It was decided that 300 female pups branded per year at each site would provide the adequate data for this study. However, due to the level of rookery disturbance and individual handling disturbance (females can only by identified after capture and close inspection of each pup) it was determined that 300 pups total (both sexes) would provide sufficient statistical precision while still minimizing wide-scale disturbance to the population. Statistical analyses of data collected from animals marked between 2001 and 2005 have shown these sample sizes to be sufficient to have high resight probability and robust survivorship estimates within 3 years of survey.

Much of our work is based on research avenues recommended in the Steller Sea Lion Recovery Plan of 1992 (and supported in the current draft 2006 Recovery Plan), and is all designed to be directly applicable to the formation of informed management decisions regarding the protection of Steller sea lions throughout their US distribution. The data this research provides is used directly in legal decisions pertaining to management of fisheries removals through citation in Biological Opinions produced by the NMFS.

C. <u>Methods</u>

1. Duration of the Project and Locations of Taking:

A five-year permit is requested for the term of June 1, 2007 through May 31, 2012. Population dynamics research will occur in all months and seasons but will be concentrated in the summer months of May-August. Resighting work must occur during the pupping season to provide the needed demographic information concerning birth and survival probabilities. Pups are born only from late-May through mid-June; median pupping dates were 4 and 9 June at Forrester and Marmot Islands (Pitcher et al. 2001). Because pups are born only during this short season, annual estimates of survival require resighting around the birth time (May-August). True birth probabilities (not confounded with pup mortality and weaning probability) can only be estimated during the pupping season. Most boat-based resighting work for survival and reproductive rates will occur when pups are > 2 wks - 2 mos of age, when pups are past the perinatal period. Resighting work at Forrester Islands only will occur throughout the pupping season from late May and include the perinatal period so that actual birth rates (not confounded with pup mortality) and pup mortality rates can be explicitly estimated during the pupping season. Pup mortality during the pupping season must be monitored to prevent bias in birth probabilities as mortality of very young pups can be significant (Kaplan 2005). Potential locations of pup branding at natal rookeries in the eastern DPS include branding at 4-5 rookeries (Forrester Island, Graves Rock, White Sisters, Hazy Island, Biali Rocks) per year, with 1-3 years skipped (no pup branding at natal rookeries) between branding years, or branding of 2-3 rookeries per year as was conducted from 2001-2005 (Table 3). To provide the needed sample size and allow natal rookery of pups to be accounted for in survival models, it is necessary to brand pups on rookeries from end June – early July. Natal rookery is

needed to account for variation in survival probabilities as first and second year survival of Steller sea lions varied among natal rookeries from 2001-2005 (ADFG unpublished data). At this time, most pups (<5% of the pups captured for branding on rookeries from 2001-2005 were <20 kg, and therefore potentially very young or starving animals; ADFG unpublished data) are 3-4 weeks of age, past the perinatal period and less vulnerable, but also not yet sufficient swimmers such that large groups of pups can be found on shore at their natal site. An example of these specific locations can be found in our most recent annual permit report for #359-1769 (refer to 2005-2006 ADFG Annual Permit report Appendix B). Announcements will be sent to NMFS prior to this work noting the location of the proposed captures. In the eastern DPS, brand-resighting will be conducted at all rookeries and haulouts in Southeast Alaska with potentially additional resighting effort in British Columbia. Sea lions marked in the Forrester Island complex are frequently observed in British Columbia. Brand-resighting may also be conducted in the western DPS in conjunction with capture work or the extension of eastern DPS studies (*i.e.,* reproductive rate and weaning studies; see below). Captures of Steller sea lions for health and condition monitoring and for foraging ecology research may occur during all months, focused in up to 4 capture trips total per year, distributed between the eastern and western DPS. Juvenile (defined for this application as animals 2 months to 3 years of age) and adult (defined for this application as animals greater than 3 years of age) captures may occur at any known or potential Steller sea lion haulout or rookery in coastal Alaska. It is difficult to determine the age or sex of a sea lion prior to capture and close examination, particularly while working underwater. Age classifications have been grouped to account for ages of animals that are likely to be captured during different capture scenarios. For instance, due to the behavioral ontogeny of sea lions it has been our experience during the past 5 years using the underwater capture technique that it is very unlikely for animals over the age of 3 years to interact with divers underwater. Final assessment of age will be made for animals up to 24 months of age by measurement of the canine length while under anesthesia. For animals estimated to be over 24 months of age a pre-molar tooth must be extracted and sectioned for age estimation (see methods for *f. tooth extraction* below). There is mounting evidence that some young sea lions may be at least in part dependent on maternal nutrition for up to 3 years, and that there may be differences in age at weaning both between years and between regions, thus division of this grouping of animals into categories of suspected nursing and weaned prior to capture and analysis are supposition at best, and possibly misleading at worst.
All capture activities will be closely coordinated with the NMML and the Alaska SeaLife Center (ASLC) to ensure that the timing of capture trips in the western stock are planned to gather the most effective seasonal distribution of data and minimize any disturbance impacts on the western DPS. ADFG operates within the Alaska Maritime National Wildlife Refuge under the authority of a Special Use Permit from the Alaska Maritime National Wildlife Refuge, Homer, Alaska and ADFG has also received an annual permit from Glacier Bay National Park (GBNP) to conduct resights and brand pups within the GBNP. Research activities are reviewed by both agencies to ensure that they are consistent with the protective status of the sites.

2. Types of activities, methods and numbers of animals or specimens to be taken or imported/exported

This permit application includes many different kinds of takes within a wide variety of inter-dependent research programs and activities. All takes requested in this application occur in wild-caught animals. Types of takes include the following:

a. incidental disturbance during aerial surveys;
b. incidental disturbance during brand-resight efforts and general observations;
c. incidental disturbance during scat collections, disentanglements, and captures;
d. potential capture of pups on rookeries for marking, measuring, and sampling;
e. capture of pups, juveniles, and adults on land, underwater, on floating pens or at-sea nets for marking, measuring, sampling and instrument deployment;
f. collection of blood, skin, muscle, vibrissae, hair, nails, stomach contents and blubber samples;
g. swabbing lesions and/or orifices (such as eyes, genitalia, or rectum) of animals exhibiting symptoms of disease and as general condition indices and collecting fecal loop samples;
h. tooth extractions;
i. injection of sterile solutions for assessment of water and blood volumes;
j. metabolic rates assessments; and
k. hot-branding of all age-classes.

For purposes of clarity, research activities are presented separately below with a narrative explanation, and summarized in Table 2. Take activities are organized as authorized in past permits and amendments for the same research (#358-1769).

<u>Descriptions of each activity as listed in Table 2.</u>

Activity 1: aerial surveys during the non-breeding season at Alsek and Akwe River mouths (disturbance level: none to very low)

<u>Objectives:</u> The final Recovery Plan (NMFS 1992) identified the need to determine seasonal use patterns of sea lions (research task 12). Aerial surveys conducted during the non-breeding season, using the same protocols and methods as the breeding surveys, are an effective tool for assessing seasonal changes in the distribution and relative abundance of sea lions. In past years large numbers of Steller sea lions have been seen congregating at various places in Southeast Alaska during ephemeral concentrations of prey species. This is especially apparent in the Alsek and Akwe river mouths near Yakatat. Aerial surveys of this area by Co-investigators with the USFS have indicated that up to 1,520 sea lions may use this area briefly to prey on eulachon (*Thaleichthys pacificus*), thus we request takes for up to 2,000 individuals in the eastern DPS, up to 10 times per year for this activity, resulting in a total of 20,000 takes some of which may be repeated takes of the same individuals (Table 2).

<u>Methods:</u> Survey protocols use a Cessna 206 or 185 fixed-wing flying at an altitude of 150-180 meters and using a Sony Mavica digital camera with a 3X focal zoom and a 20X digital zoom to photograph hauled-out sea lions. Surveys are conducted up to 8 times per year between February and May. The images are downloaded into an IBM computer processed with Paintshop Pro™ and counted by creating a point theme in Arcview GIS 3.2. The photos are counted three times, each count saved as an active theme and the average of each count is used as the total count for that survey. Survey flights are flown during mid-day between the hours of 1000 to 1400 when sea lions are most likely to be hauled out (Pitcher, ADFG, personal communication). To estimate relative abundance, all sea lions observed during a survey flight are counted; a maximum of 1520 sea lions have been hauled-out at these sites in these months.

Expected Results: Data from non-breeding surveys, particularly when compared to results of previous surveys, will help describe and identify seasonal movements of sea lions in Alaska. This information will be especially important for assessing the potential impacts of winter fisheries on the prey resources of sea lions.

Activity 2: Brand-resighting and associated observations (disturbance level: very low to low)

Objectives: The objectives of resighting branded sea lions include estimating important life history parameters including age-specific survival, weaning, and reproductive rates; and describing distribution and movement patterns based on sex, age, and natal rookery. Additionally, observing previously-handled sea lions provides a means to evaluate impacts of studies requiring handling as well as yielding information on the weaning status of previously studied animals.

Methods: Observations of branded or other previously marked sea lions are accomplished from land, skiff, or ship. We request takes of up to 24,500 individuals in the eastern DPS, up to 3 times per year, plus up to 500 individuals in the Forrester Island complex, eastern DPS up to 20 times per year and up to 5,000 individuals in the western DPS up to 3 times per year for this activity, resulting in a total of up to 25,000 takes in the eastern DPS and up to 5,000 takes in the western DPS, some of which may represent repeated takes of the same individuals (Table 2.). In all cases, approaches are conducted to minimize disturbance (or in the case of land locations, almost always without being observed or detected by sea lions). Extreme care will be used on first approach to haul-outs and rookeries by skiffs such that at >200-400 m, animals will be given at least several minutes to notice our presence without further approach. The skiff will remain at this distance stationary or moving at < 5 mph. This will prevent most agitation of animals as Steller sea lions react most strongly when approached unexpectedly and at high speeds by skiffs. Only if we observe animals are behaving naturally and appear to be comfortable with our presence (not looking at the skiff and altering their movements on land or moving towards the water) will the skiff approach closer at ≤ 5 mph. Only as long as animals continue to appear comfortable with the presence of the skiff, most skiff-based resighting work will be conducted at a distance of 10-50 m from the shoreline. If animals remain uncomfortable with our presence, resighting will be conducted at a distance where few or no animals appear agitated. Observations from ship-based platforms will be conducted infrequently (usually only when sea conditions prevent launching skiffs) at minimum distances of 100 m. Land-based observations will be conducted at various distances depending on topography of sites. At ~20-40% maximum of sites visited by skiff, will observers also resight animals from shore from hidden locations at distances of 50 – 200 m. Visual observations of brands and marks are made using binoculars (often image-stabilized) or spotting scopes, and digital images are obtained of most (>97%) of the marked animals observed during dedicated resighting efforts. Observers' interpretation of marks are recorded in notebooks, compared to digital images, and entered into an ACCESS database. A photo library containing images of all ADFG-marked animals is used to confirm resight observations. Photos and resighting data are shared quarterly among all groups marking and resighting Steller sea lions (National Marine Mammal Lab, Oregon Department of Fish and Game, Alaska Sea Life Center, Russian and Canadian counterparts), using a standard or comparable format for all groups to maintain libraries of their marked animals. Additional data collected during resight efforts include: a count of non-pups, a count of pups (except at larger rookeries, where accurate pup counts are especially difficult and time consuming to obtain), and a description and photograph of sea lions with injuries, rope/net entanglements, or ingested fishing gear (as indicated by fishing line, hooks and flashers with line protruding from their mouths).

The ADFG will conduct the following dedicated resight projects per year in Southeast Alaska in the eastern DPS: (1) annual summer survival and reproductive rate survey(s): conducted daily for 4-8 weeks during summer (May-August) at haul-outs and rookeries throughout Southeast Alaska, including multiple visits to selected haulouts and rookeries (2-5 visits per summer at 1-25 sites, 2-20 visits per summer at 1-6 sites) to improve resighting rates of juveniles and at rookeries (1-5 days per rookery per trip) to estimate reproductive rate and weaning probabilities via a robust design (Kendall et al. 2004). (2) Resight surveys in winter and spring months to estimate age-specific weaning probabilities and other parameters from October through May for 5-7 days per month within a 14-day period at 4-8 haul-outs. (3) Reproductive rate surveys at Forrester Islands for 1-3 consecutive days (grouped survey) every 1-2 weeks from May 1 – July 30, and possibly until September 30 (minimum interval between grouped surveys of 5-7 days). (4) Miscellaneous resight surveys year-round, also including surveys during trips primarily intended for animal captures. Depending on availability of funding, the ADFG may conduct brand resight surveys at rookeries and haul-outs in the western DPS, including boat and land-based surveys for reproductive rate estimates (up to 3 consecutive days per rookery for up to 5 rookeries; or 1-3 day surveys every 1-2 weeks from May 1 – July 30, at 1-2 rookeries per year), age-specific weaning probabilities (October through May for 5-7 days per month at 4-8 haul-outs per year), and miscellaneous resight surveys year-round, also including surveys during trips primarily intended for animal captures. The number of disturbances per individual per year is unknown.

Expected Results: Observations of branded or marked sea lions will continue to provide estimation of age-specific survival, weaning and reproductive rates, and dispersal and distribution patterns, information critical for comparison with the endangered western DPS.

Activity 3: Incidental disturbance during scat collection and capture/sampling, activities (disturbance level: moderate)

Objectives: This activity also accounts for disturbance incidental to accomplishing scat collections, and capturing and sampling of all age groups (Activity 7). We request takes of up to15,000 individuals in the eastern DPS up to 3 times per year and 2,000 individuals in the western DPS disturbed up to 4 times per year for this activity. The Recovery Plan for Steller sea lions (NMFS 1992) identified the need to investigate feeding ecology and factors effecting energetic status (research task 6) and specifically to describe foods eaten by sea lions (research task 611) and to assess significance of various prey (research tasks 614). Likewise the draft Revised Recovery Plan (NMFS 2006) identifies continued similar data needs (task 2) including the need to continue to estimate survival, fecundity, and immigration/emigration rates through a brand-resight program (task 1.2.1). Evaluation of the hypothesis that food limitation plays a major role in the decline of sea lions in the western stock requires knowledge of food habits by area, season, and sea lion age class. Our primary source of food habits data comes through identification of prey remains in fecal material (scats) collected at rookeries and haul-outs. In the past, ADFG has participated with scientists from the University of British Columbia to collect scats during our annual resight trip. The geographic and temporal scope of scat collections has increased as the number of sites visited for resights has increased. The objective of scat collections is to fill in data gaps regarding seasonal and temporal differences in prey use.

Methods: Sea lions will be cleared as slowly as possible to avoid causing stampedes or panic, by researchers landing on shore in an area hidden from animals if possible, and slowly and cautiously approaching sea lions, stopping movement and hiding when animals appear too agitated (moving quickly or stampeding into the water). Occasionally, skiffs near shore are used to encourage animals to move into the water at a controlled pace (by altering the distance from shore and speed of the boat to either encourage or slow movement). Scats will be opportunistically collected when rookeries and haulouts are disturbed for animal captures, or in conjunction with resight activities only in rare cases when all animals are unintentionally and inadvertently disturbed into the water. Scats are individually bagged, tagged, and shipped to UBC in Vancouver, BC, Canada. Prey remains are identified by Pacific ID and hard parts (typically cephalopod beaks, bones, and otoliths) are then identified to taxa.

Methods used in captures are described under Activity 7. Sea lions may be taken under this activity more than once per year, and may also be taken under Activities 1, 2, and 3.

Expected Results: Scat analysis provides a description of the frequency of occurrence of various prey items by area and season. These food habits data will then be compared with historical data on prey preferences and prey availability to evaluate the hypothesis that changes may have occurred in prey consumption.

Activity 4 & 5: Collect carcasses or parts of carcasses of Steller sea lions and receive tissue samples from subsistence-harvested sea lions (disturbance level: none to moderate)

Objectives: The Recovery Plan for Steller sea lions (NMFS 1992) identified the need to investigate carcasses (task 5) and collect samples from natural death as well as subsistence harvest. In addition, grantees from NMFS-funded grants have requested various tissue samples from carcasses of all age classes for investigations of disease, histology, parasitology and other indices of condition.

Methods: When available, carcasses will be collected in whole for transport to a laboratory. When in the field, tissue samples will be collected and preserved according to set necropsy protocols. A Memorandum of Agreement has been set up between the ADFG Steller sea lion research program and the Aleut Community of St. Paul Island to allow subsistence-harvested tissues to be collected and sent to our laboratory. Additionally, the ADFG may conduct surveys in Southeast Alaska in the eastern DPS, dedicated to counting and recovering aborted fetuses during the months of Dec – May, when most aborted fetuses have been observed. For example, provided money is available, this effort may be undertaken in the event of frequent sightings/reports of aborted fetuses on haulouts Four to eight haulouts may be visited as frequently as twice per week to search for aborted fetuses using binoculars from skiffs or hidden shore sites that are >50 m from animals. Animals will only be disturbed during these surveys when fresh, recent-aborted fetuses (as observed remotely using binoculars) are observed in areas of the haul-out which would require minimum to no disturbance to collect (on the edges or outlying areas near haul-outs, or retrieved from in the water near haul-outs). ADFG has successfully retrieved fresh fetuses from the water near haul-outs without modifying the behavior of animals hauled-out. Disturbance to animals will be minimized, with the haulout approached slowly to avoid a panic or stampede. If a haulout is disturbed by a fetus being collected, that site will not be disturbed again for at least one week (in some situations, a fetus can be collected without disturbance to hauled out animals). Depending on the age of the carcass, it will be shipped either chilled or frozen to a veterinarian or veterinary pathologist for subsequent analysis.

Expected Results: Analysis of fresh dead carcasses could provide a rare glimpse into the causes of mortality of Steller sea lions. Carcasses are extremely rare and as such, very valuable from a scientific perspective. Subsistence-harvested animals allow us to collect very fresh samples for use in cell cultures and virology investigations and can also yield information on the baseline level of condition indices in an otherwise healthy animal.

Activity 6: Incidental mortality (disturbance level: very high)

Objectives: This Activity provides for accidental mortalities that may result from other research activities. Up to 10 mortalities per year may occur (not to exceed 5 per year in the western stock) during this research. In the past 3 years, except for two mortalities (a juvenile female that died under anesthesia and a juvenile male that died of asphyxiation under NMML permit), all mortalities have been pups < 2 months of age and occurred during the moving of pups or anesthesia for branding.

Methods: Not applicable. To the extent possible, necropsies will be performed on and samples collected from carcasses resulting from accidental mortalities.

Expected Results: Not applicable. However, necropsy results should provide insight into mechanisms of death, yield access to extremely rare and fresh tissue samples, and an opportunity to make other measurements of health and condition that would not otherwise be available from live captured individuals.

Activity 7: Capture (includes hand, hoop net, underwater noose, floating trap, at-sea net capture, dart injection) and morphometrics (disturbance level: high)

Objectives: The Recovery Plan for Steller sea lions (NMFS 1992) identified the need to monitor the health, condition, and vital parameters of sea lions, and this need has been reiterated by subsequent reviews and the draft 2006 Recovery Plan to facilitate testing multiple hypotheses for the sea lion decline. Health and condition of the Alaskan sea lion population is important for monitoring the population status with respect to recovery, and for determining causes of the population decline. Juvenile survival may be an element in the continuing decline and will be critical to the recovery of the western stock of Steller sea lions. Availability and quality of food resources is a likely mechanism for influencing juvenile survival, especially during the winter. Assessing the condition, status, and foraging behavior of pups as they are weaned and of juvenile sea lions that are foraging for themselves is the most direct means to understand this critical time in a sea lion's life. More specifically, with the respect of the draft Revised Recovery Plan (NMFS 2006) this includes developing indices of health and body condition (task 1.3.2), developing improved live capture techniques for research needs (task 1.4.1), obtaining measurements and samples using non-lethal techniques (task 1.4.2), marking of pups on rookeries, continue ongoing studies of the physical condition of pups on rookeries, and of pups and juveniles outside of the breeding season, particularly during the winter.

The Recovery Plan for Steller sea lions (NMFS 1992) and the NRC (2003) identified the need to identify habitat requirements and areas of biological significance for Steller sea lions and to investigate feeding ecology. Specific points include: deploying instruments to obtain finer scale data on sea lion foraging habitat (task 2.3.3), to map, describe, and evaluate feeding areas and needs (task 2.3.4), determine seasonal use patterns, refine understanding of Critical Habitat use, assess the relationships between oceanographic features and sea lion foraging ecology (task 2.4.1), and investigate diving behavior/physiology and feeding cycles (task 2.5.1). Participants in a telemetry workshop convened by the Recovery Team in December 1997 reiterated the importance of telemetry studies, especially those targeting feeding ecology and movements of juvenile sea lions.

A number of studies have been developed to obtain better measurements of health and condition, at-sea behavior, diet, and survival. All require capture, restraint, and sampling of sea lions. Capture, restraint, and external morphometric measurements are described for Activity 7 in general. Specific study methods for marking, tagging, and sampling those animals that have been captured have been subdivided into sub-Activities 7a-p. Sea lions taken under Activity 7 may also be taken under Activities 1, 2, 3, 5, and 6.

All activities are coordinated with research activities of the National Marine Mammal Laboratory, Alaska SeaLife Center, University of Alaska, Aleutians East Borough, University of Washington, Texas A&M, Oregon Department of Fish and Wildlife, Washington Department of Fish and Game, and other researchers to minimize disturbance. There are instances when researchers from different agencies will conduct studies at the same haulout or rookery location. Communication and coordination between agencies has assured not only that disturbance of animals at a particular location is distributed amongst different times of the year, but also that cooperative research opportunities are maximized to decrease the activity at each haulout and maximize the amount of information that can be gained from each known (marked) animal. One example of this cooperation is cooperative research cruises such as the ones conducted in February 2005 by ADFG and ASLC in Prince William Sound, and in April 2005 by ADFG and NMML in the Aleutian Islands. Prior to each capture trip researchers at other agencies are consulted to agree upon whether previously studied and marked animals should be targeted for continued study on a case by case basis. For instance, incredibly valuable information can be gained from measuring body condition and identifying current diet of marked animals to assess growth rates and time of weaning in different segments of the population. However, in some cases, previously studied animals will be rejected for capture, such as a recently released sea lion from the ASLC transient program.

<u>Methods:</u> Captures are accomplished by several techniques that differ for targeted age classes. Pups (newborn to 2 months old; since presence of umbilicus and estimation of age cannot be assessed until the pups are closely examined after capture) are captured by hand, juveniles (>2 months to 3 years old) are captured by hand, or by using hoop nets, an underwater noose, a floating pen, or an at-sea net capture technique, and adults (>3 years old) are captured using floating pens, the at-sea net capture technique, or remotely-delivered (darting) chemical anesthetic. Restraint techniques similarly vary by age class. Pups (< 2 months old) on rookeries are restrained by hand or by using gas anesthesia (if hot-branded under Activity 7.i), whereas juveniles (2 months to 3 years old) are restrained physically (hand or restraint device) or chemically (valium or gas anesthesia). Adults are restrained physically (squeeze-cage), chemically (valium), with gas anesthesia, or a combination of the above based on judgment of the attending veterinarian. A veterinarian will be present if chemical immobilization is used.

Up to 800 pups aged newborn to 2 months old in the eastern DPS will be captured by hand, weighed, measured and branded each year (Table 2). Fifty pups per rookery (approximately 25 males and 25 females) provides a minimum sample size useful for statistical analyses of mass by sex class (sufficient to detect a 15% change in mass of males and a 10% change in the mass of females [α=0.05 and statistical power ($1-\beta$) = 0.80]. Pups are captured by hand, placed in a hoop net for weighing, and restrained with gas anesthesia for measurement, sampling, and branding. Measurements include mass, standard length and axillary girth (immediately behind fore flippers). Pups that are not hot-iron branded (Activity 7i) will be measured and then marked or tagged for future identification using only physical restraint (Activity 7h). Gas anesthesia (isoflurane) reduces stress on pups and improves the quality of brands by preventing wiggling during branding. We use equipment and techniques developed and described in detail by Heath et al. (1996) and Heath et al. (1997). This technique has been used extensively with Steller and California sea lions, both adults and pups, and was in fact developed primarily for and during field operations on these species in collaboration with the NMML and the ADFG. We will deliver anesthesia to hand-restrained pups through a mask, sufficient for the time requirements of branding (typical anesthesia administration time is 8 to 12 minutes at a surgical plane, although under situations of slow induction and/or extensive sampling this can reach a maximum of 25 minutes). Gas anesthesia will be administered and monitored only by personnel thoroughly trained in its application.

Older pup and juvenile (>2 months through 3 years) capture techniques will vary by size of animal and location of capture. Up to 250 sea lions of this age group (100 in the eastern DPS and 150 in the western DPS) may be captured per year. Juveniles on land may be captured with a large hoop net (3 ft. diameter and 5 ft. long handle). This method involves up to three biologists sneaking up as close as possible to the target animals before entrapping them in nets. This technique has proven safe for both researchers and animals and has only had one recorded mortality during a capture trip. Hoop-net captured juveniles may be physically restrained by slowly transferring them into fabric restraining wraps used for weighing. Sea lions are restrained in this wrap during measurements and sample collection. Injectable valium or isoflurane gas may be administered under veterinary supervision to reduce struggling. However, land-captured sea lions will be preferentially transferred to a research vessel and restrained with isoflurane, as are sea lions captured with underwater noosing. Sea lions are inducted using masks, then endotracheal tubes are inserted (Heath et al. 1997) to continue administration during sampling procedures. This age group is also successfully captured in the water using the lasso technique (Raum-Suryan et al. 2004). Two or three divers, supported by skiffs and a larger vessel, approach a haulout under water. The natural curiosity of young sea lions draws them to the divers. After a brief period, sea lions will approach close enough that a rope lasso can be placed over their heads by the divers. The lasso is tightened on the lower neck/shoulder region and the rope is retrieved by the crew of the second capture skiff. Animals are wrapped in a restraining net, pulled into the skiff using the net and placed directly into padded, ventilated capture boxes. As soon as the lid to the capture box is secure the lasso is removed from the neck/shoulders of the animal. This technique has proven to be effective and safe for divers and captured animals, and has not resulted in an injury or mortality by either the NMFS or ADFG. It is also possible this age class will be captured by floating trap (pens) or at-sea net capture technique (described below). Once restrained, measurements of weight and size (lengths, girths, teeth measurements) are taken and samples obtained as described in 7a-p below.

Platform traps consist of a buoy with a 12-foot square platform for a haul-out surface and a 6-foot high steel cage perimeter, similar to traps that have been used to capture California sea lions

(*Zalophus californianus*) and Steller sea lions in Washington (under Permits No. 835 and 782-1446). These platforms remain open for voluntary movement of animals until a research team is in attendance and ready to capture and handle animals. As many as 20 sea lions can be captured in the traps at a time, but only 5-6 individuals have used one of the new traps (Kodiak) thus far. However, this number is expected to increase as sea lions acclimate to the trap. Once sea lions are captured, they are individually transferred to, restrained, and sampled (average time ~ 30 minutes) in a stainless steel squeeze cage that restricts movement without the need for immobilizing drugs. The squeeze cage is adjacent to the trap, allowing personnel to monitor the remainder of the animals in the trap. Duration of time in the platform trap will depend upon the number of animals captured, however will be less than a maximum of 12 hours during which time researchers are sampling each sea lion captured. At least two locations will be utilized for floating trap captures in collaboration with the NMFS; Kodiak harbor and Resurrection Bay (both within the western DPS). Other sites may be used if logistically possible. Juveniles and adults captured in floating traps will be restrained physically by means of a squeeze cage. Additional restraint with isoflurane administered as above may be used if appropriate. Adults may also be captured by use of drugs delivered via propelled darts. After stalking as close to a target sea lion as possible, a dart is fired to deliver drugs *intra muscularly* (IM) preferentially over the hips and tibia lumbar muscle, or into muscle over the shoulders (Haulena and Heath 2001). Telazol (Tiletamine and Zolazepam) is delivered at dosages of 1.8-2.5 mg/kg (Loughlin and Spraker 1984), although Medetomidine with ketamine or telazol may also be used at the discretion of the attending veterinarian (Haulena and Heath 2001). Adults captured by darting may be additionally restrained with isoflurane gas, at the discretion of the attending veterinarian depending upon environmental conditions and the duration of sedation required.

Limitations with the trap captures and noosing technique have resulted in a paucity of data on the foraging behavior of sea lions > 3 years old, a group for which far more information is needed so potential effects of commercial fishery management measures can be evaluated (Capron and Fritz 2004). Thus, an at-sea net capture technique, which was developed by Simon Goldsworthy (La Trobe University, Victoria, Australia), will be used to capture larger juvenile and adult Steller sea lions in remote areas of Alaska. This technique was originally devised to investigate Australian sea lion (*Neophoca cinerea*) interactions with commercial fisheries. Because the interactions occurred in specific locations, rather than capturing sea lions on the beach and hoping the animals would go to the fishery grounds, Dr. Goldsworthy captured sea lions at-sea that were directly interacting with the fleet. Australian sea lions are attracted to catcher/processor vessels, so the capture team worked on board one of the commercial vessels during the fishing season. A pouched net of trawl mesh is strung from a square frame and fish are attached to the bottom of the inside netting (Figure 1A). The net assembly is lowered adjacent to the vessel using a boom (Figure 1A) and suspended just beneath the water surface, and sea lions become interested in getting to the fish at the bottom of the net (Figures 1B and 1C). When a single sea lion is at the bottom of the net, the boom operator lifts the net out of the water (Figure 1D), and the sea lion is brought on board (Figures 1E and 1F). The sea lion is then sedated with an injectable drug, lowered into a capture box, anesthetized with an inhalable agent, outfitted with a transmitter, allowed to recover and enter the water down the trawl ramp. This has proven to be a very successful technique and the only mortality that occurred during this procedure was drug-related (due to the injected sedative).

We believe this technique is directly transferable to Steller sea lions. Fishermen and fisheries biologists report that Steller sea lions approach vessels trawling or processing catch during certain times among some fisheries in the Aleutian Islands and Bering Sea. These sea lions attempt to take fish from the gear or offal that is flushed overboard during processing. Based on observations and incidental catch data, these sea lions are most likely older juveniles and adults. We will construct a net of trawl mesh strung beneath a square metal frame, using a mesh size sufficiently small to preclude insertion of sea lion noses or heads, and of material strong enough to support the weight of juvenile to adult sea lions. Fish obtained directly from the fishing vessels or from shore-based processors will be attached to the bottom of the net (for example, cod, pollock, Atka mackerel, salmon). Though we may attempt captures directly from fishing or processor vessels, we currently plan to operate from chartered research vessels to minimize interference with fishing operations and accommodate sea lion handling and sampling. Thus, in areas where sea lions are interacting with fishing gear or processors we will lower the net via a boom, either from the fishing vessel or from a vessel nearby the interaction. The net will be submerged just beneath the surface to a depth encouraging entrance by sea lions, but allowing visibility to the bottom of the net. When a sea lion is inside the net structure, the boom operator will lift the net with sea lion out of the water, and transfer it to the deck of the ship. The sea lion will be placed into a capture cage as is done for underwater noose captures, or placed within a pen (on the deck) and directed into a squeeze cage as is done for floating platform captures.

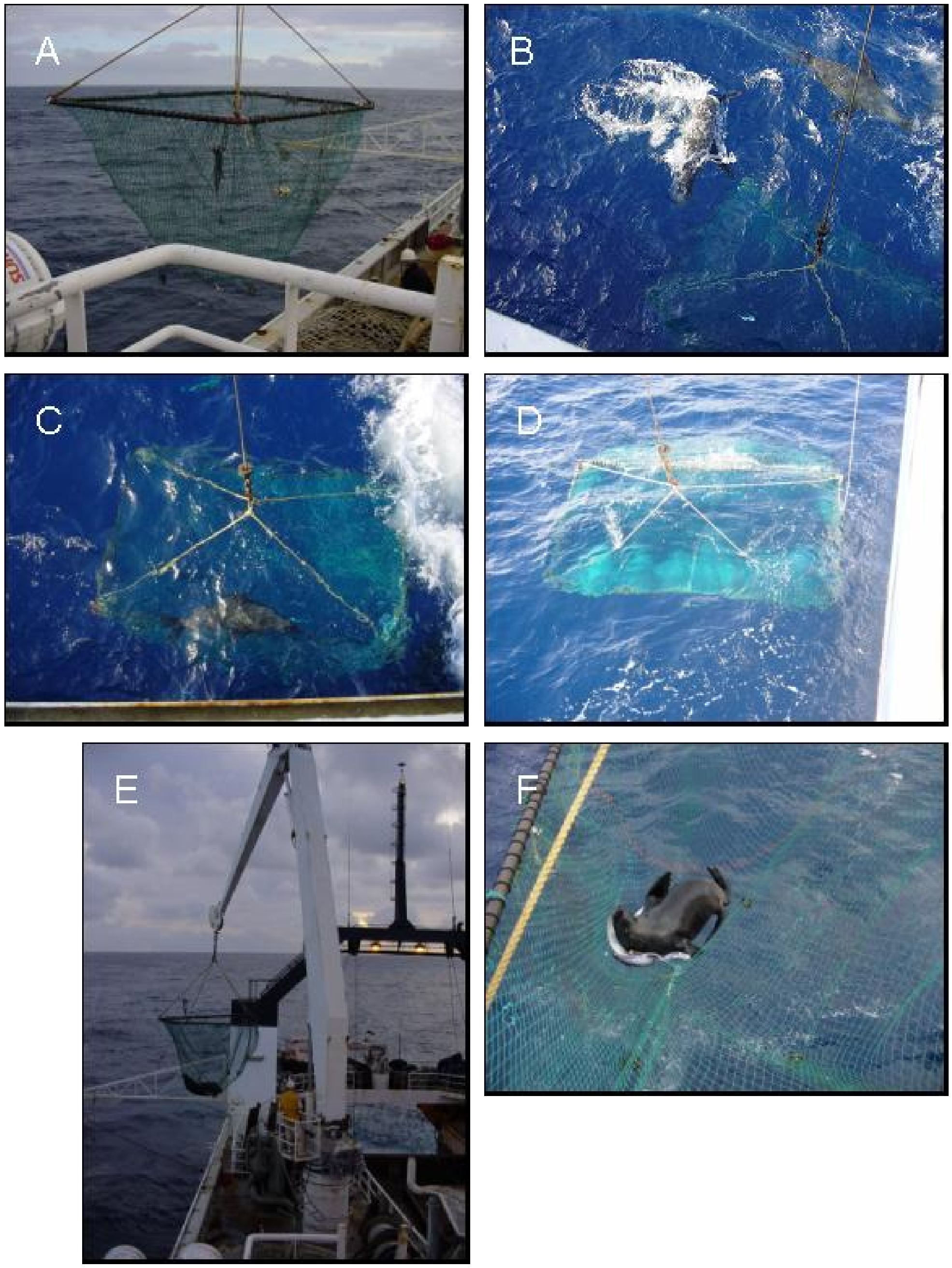

Figure 1. Series of photos depicting at-sea net capture process of Australian sea lions. A) net assembly is lowered over side of ship; B) sea lions swim around submerged net; C) sea lions dive into net to retrieve fish; D) net is then hoisted; E) net with sea lion is boomed aboard; F) sea lion in net. Photographs by Simon Goldsworthy

The following procedures may be performed on captured sea lions, as enumerated in Table 2.

a. Blood collection. Blood samples will be taken from up to 350 pups (<2 months old in the eastern DPS) captured on rookeries during the breeding season, from up to 250 juveniles (>2 months to 3 years old; up to 4 times per year; 100 in the eastern DPS and 150 in the western DPS) and from up to 30 animals >3 years old (up to 2 times per year; 15 each in the eastern and western DPS; Table 2). Clinical blood chemistries are useful for examining the gross physiological status of individual animals. In other otariid species, electrolytes, oxygen carrying capacity (red blood cell indices), and immune system function (white blood cell indices) are highly useful for making inferences about the health of young animals. The NMML began analyzing blood from Steller sea lion pups in 1991. More thorough and regular monitoring of the clinical chemistries of pups began in 1998. Based on the observed variability in the samples from 1998 and 1999, samples sizes necessary for detecting differences between each rookery are prohibitively large. However, comparisons between rookery groups (e.g., Southeast Alaska, Gulf of Alaska, etc.) and between years requires approximately 85 samples per year for detecting differences in hematocrit, and 120 to 160 samples per year for detecting differences in most electrolytes [for $\alpha=0.05$ and statistical power $(1-\beta)=0.80$]. Blood draws will be made by personnel trained/experienced in blood draw techniques. Blood will be collected from the caudal gluteal vein (18 g x 2 1/2 " spinal needles) or hind flipper vein (21 g x 1" butterfly). 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. The area to be sampled will be thoroughly disinfected with ethyl alcohol or betadine prior to insertion of the needle. Each needle may be reinserted into the skin once if the first site does not work but only if not dull and not contaminated by handling. A maximum of three needle insertions will be made at any one site at any one session to reduce muscle injury and bruising and to reduce risk of introducing infection. Sufficient pressure and/or dry gauze will be applied to the venipuncture site after removal of the needle to minimize the potential for hematoma formation in the surrounding tissues. This procedure will only be performed by/under the direct supervision of qualified and experienced personnel.

A total of not more than 1ml of blood per kg of body mass is taken from any animal per capture event (typically no more than 120 ml total, depending on animal size). Blood is drawn at three times, once prior to deuterated water (DTO) administration, and at two subsequent times (20 minutes apart) once the DTO is equilibrated with the animals blood pool (approximately 2 hours following DTO injection). When Evans Blue is being used for blood volume analysis, post DTO blood samples are drawn from a catheter placed in the hind flipper vein. Evans blue dye is injected through this catheter and 3 subsequent blood samples are drawn at 10-minute intervals from the catheter. Blood samples are stored at -10C or on dry ice after processing while at sea, shipped on dry ice and stored at -80C upon return to the laboratory until analysis. A minimum of 37 ml of serum (roughly equivalent to 75 ml of whole blood depending on hematocrit) is needed to perform the following analyses: hematology (hematocrit, hemoglobin, specific gravity, plasma and whole blood water content), clinical blood chemistry, fatty acid composition, deuterium analyses, metabolic chemistry, serology, virology assays, stable carbon and nitrogen isotope content, haptoglobin, Evans' blue concentration, sodium bromide concentration, serum iron content, growth hormone and dietary bioindicators (e.g. TMAO). Any additional serum or plasma is archived for future retrospective analyses and sample requests made by other researchers. If less than 75 ml of whole blood is drawn, then a subset of these analyses are prioritized. Blood chemistry is analyzed by commercial veterinary clinical lab or in our ADFG/UAF laboratory.

b. Muscle biopsy. Muscle oxygen stores are determined by measuring muscle myoglobin content and estimating muscle mass (from deuterated water estimate of lean body mass and allometric equations). Myoglobin content will be determined following methods widely applied to pinnipeds (including Steller sea lions) without adverse effects or complications (Kanatous et al. 1999; Ponganis et al. 1993; Reed et al. 1994). Additional muscle structure, hormone and enzyme activity analyses will be performed on the samples to examine factors that influence muscle structure and function (Castellini and Somero 1981; Reed et al. 1994; Kanatous et al. 1999). A small muscle biopsy is taken from the major swimming muscle (pectoralis) and the longissimus dorsi (used for terrestrial support and locomotion). On sea lions inducted with gas anesthesia (250 juveniles, > 2 months to 3 years, 100 in the eastern DPS and 150 in the western DPS; 30 adults, > 3 years of age, 15 each in the eastern and western DPS; all up to 2 times per year), sampling sites will be scrubbed with Betadine and alcohol, and a small incision (large enough to accommodate the biopsy punch, approximately 10 mm) made with a sterile scalpel blade. Using sterile techniques, a sterile biopsy punch of 6-8 mm diameter will be inserted into the incision and pushed through to the muscle layer fascia. The punch is then rotated and extracted with the muscle sample and pressure applied to the site. Up to two 25-35 mg samples are taken from each site at different angles. Two samples are necessary because myoglobin assays require flash frozen tissues, while the fiber type assays require fixed tissue. The consensus of marine mammal veterinarians is that under these conditions, it is preferable to not close the biopsy sites with surgical clamps or sutures (Dr. Kimberlee Beckmen, pers. comm.). Closing the site increases the risk of infection and abscess formation. The longissimus dorsi biopsy will be taken from the blubber biopsy site (see 7d.) to reduce the number of incisions made. Samples are stored on dry ice while at sea, shipped on dry ice and stored at -80C upon return to the laboratory until analysis. Muscle samples are analyzed in our ADFG/UAF laboratory for histochemistry and morphology.

c. Skin biopsy. A sample of skin, approximately 5mm in diameter, punched from the webbing of the hind flipper for genetic analyses will be taken from all animals handled. Skin samples will be taken from up to 800 pups between > 5 days to 2 months of age in the eastern DPS, 250 juveniles between > 2 months and 3 years (up to 2 times per year, 100 in the eastern DPS and 150 in the western DPS) and up to 30 animals > 3 years of age (up to 2 times per year; 15 each in the eastern and western DPS). Samples will be preserved in a saline/DMSO or ETOH solution for future analysis of mitochondrial and nucleic DNA. In addition, biopsies will be taken of lesions if the veterinary pathologist present determines that it is of scientific importance. Most of these biopsies will be of skin lesions, possibly oral lesions. The animal will be under anesthesia already. The biopsy site will be prepped by a light wipe with isopropyl alcohol. A 6 mm sterile biopsy punch will be used for the biopsy and the sample picked up with sterile forceps. Multiple biopsies (3-4) of a lesion will be taken for diagnostic purposes. Biopsies will be split, ½ placed in formalin, ½ in a cryovial and frozen immediately for culture. Depending on the lesion, this will be submitted for viral, bacterial and, or fungal culture or PCR.

A skin scraping would be taken in any case where mites are suspected (hair loss in a patchy or extensive pattern, signs of self trauma from itching, reddened, thickened skin). The scraping will be done in the standard veterinary diagnostic technique as described. Several areas of lesions are selected, in each area, the skin is pinched up firmly between the finger tips, a dulled #10 scalpel blade with a drop of mineral oil on it is held in the opposite hand and the raised folded skin surface is scraped firmly with the dulled blade at a very oblique angle. The pinched skin is squeezed during the process to extruded mites from the sebaceous glands so they can be picked up with the skin cells on to the oily blade. The skin is scraped until the superficial epidermis is excoriated. The material scraped up onto the blade is placed on a glass microscope slide, another drop of oil added, cover-slipped, and then viewed under a microscope immediately. The slide is scanned for the presence of mites. A skin scraping is an important adjunct to biopsy and can be more sensitive diagnostically for dermodectic mange mites than biopsy which can often miss the mites and eggs.

d. Blubber biopsy. We will sample blubber from up to 20 Steller sea lion pups > 5 days to 2 months of age in the eastern DPS, from 250 juveniles 2 months old to 3 years of age (up to 4 times per year; 100 in the eastern DPS and 150 in the western DPS) and 30 adults > 3 years of age (up to 2 times per year; 15 each in the eastern and western DPS) for fatty acid and toxicology analyses by taking up to 3 biopsies to the depth of the underlying muscle layer (up to 0.5 g each, up to 5 cm depth depending on age of animal) from anesthetized animals. On sea lions inducted with gas anesthesia a single sampling site in the pelvic region will be shaved and scrubbed with Betadine and alcohol. A small incision (large enough to accommodate the biopsy punch, approximately 10 mm) will be made with a sterile scalpel blade and using sterile techniques, a sterile biopsy punch of 6-8 mm diameter will be inserted into the incision and blubber samples will be collected at 3 different angles from the incision (leaving only a single 10 mm straight incision). If not under general anesthesia, Lidocaine (1cc of 2% solution) will be injected in a rosette around the biopsy site as a local anesthetic and to reduce bleeding. Samples are stored at -10C or on dry ice while at sea, shipped on dry ice and stored at -80C upon return to the laboratory until analysis. Samples are analyzed by UAA ASET laboratory or at various collaborating laboratories such as NWFSC/NOAA for contaminants.

e. Fecal loops and culture swabs. We will collect fecal samples using a sterilized fecal loop for determination of parasites, disease, and hormone concentrations, and use sterile culture swabs to swipe dermal lesions, or ocular, rectal, and/or vaginal areas, as appropriate, from any handled sea lions for surveillance of disease. Fecal loops and swabs will be taken from anesthetized animals in the following age groups: 350 pups age > 5 days to 2 months in the eastern DPS (subset of the 800 pups in section c. skin biopsy above), 250 juveniles age 2 months to 3 years (up to 4 times per year; 100 in the eastern DPS and 150 in the western DPS), and 30 animals greater than 3 years of age (up to 2 times per year; 15 each in the eastern and western DPS). Swabs will be taken and cultured according to standard veterinary procedures. Samples are stored on dry ice while at sea, shipped on dry ice and stored at -80C upon return to the laboratory until analysis and analyzed by various collaborating laboratories.

f. Tooth extraction. For juveniles (up to 30 animals of > 2 months to 3 years from each stock) and adults (> 3 years, 15 each in the eastern and western DPS) extraction of one 2nd pre-molar tooth from the right side would be accomplished by use of a scalpel to loosen attachments, and then extracted with a dental elevator on sea lions under general gas anesthesia. Significant progress has been made over the past 5 years to develop a technique of determining age of juvenile sea lions (up to 24 months of age) using measurement of the length of the canine tooth and the diastema (King et al. in press). This statistical relationship will negate the need to pull additional teeth for aging unknown juvenile sea lions under the age of 24 months in the future. Currently this relationship can only be applied to animals up to the age of 24 months. Additional data must be collected from known aged animals (previously branded as pups) who are 24 months and older at the time of next capture to extend the application of this relationship. Until this is available, the age of unmarked sea lions estimated to be older than 24 months based on body size and tooth development will need to be determined by counting incremental growth layers on a longitudinal section of the pre-molar tooth using standard procedures at the NMML laboratory or a private contractor (Matson). Only one tooth will be extracted over the life of the animal. Teeth are stored and transported dry.

g. Pull vibrissae, clip hair and nails. One vibrissae may be pulled from anesthetized sea lions (20 in the eastern DPS >5 days to 2 months; 250 > 2 months to 3 years, up to 2 times per year with 100 in the eastern DPS and 150 in the western DPS; and 30 animals >3 years, up to 2 times per year; 15 in each eastern and western DPS) for stable isotope analysis to help identify the general trophic level at which an animal is feeding over prolonged periods. Pulling, rather than clipping, vibrissae is preferable because clipping results in an unknown length remaining attached to the sea lion, which reflects the most recent diet. Stable isotope ratios show patterns in Steller sea lion vibrissae, and changes in ratios can occur in less than 1 cm (Hirons et al. 1998). Thus, obtaining the root of the vibrissae, representing the most recent growth, for analysis is crucial. Vibrissae are pulled by gripping with forceps and pulling forcefully and rapidly in one smooth motion. From these animals we will also collect other tissue samples for stable isotope or contaminants analysis: a small sample of hair, clipped from an area approximately 3cm by 3cm (typically associated with preparation of a biopsy site), the tip of a nail from each fore flipper and ingested milk (7n.). Vibrissae, hair and nails are stored and transported dry. Vibrissae are sectioned in our ADFG/UAF laboratory and analyzed either by USGS or UAF under cooperative agreement. Hair is analyzed by cooperating laboratories such as NWFSC/NOAA or Todd O'Hara/UAF.

h. Flipper tag or other mark. In cases where branding is not used to place a permanent mark on a captured sea lion (for example at rookeries where pups are not branded because resight effort is not possible, or where land captures of juveniles or adults occur) and a short-term mark is desirable to reduce handling confusion or monitor sea lions for less than two years, alternatives to branding will be considered at the discretion of the investigator. A total of 200 pups age newborn to 2 months from the eastern DPS and 250 juveniles > 2 months to 3 years (2 times per year, 100 in the eastern DPS and 150 in the western DPS), and 30 animals > 3 years (2 times per year, 15 each in the eastern and western DPS) will be marked. Marking commonly used in pinniped or wildlife biology include double color-coded plastic flipper tags (for example made by All-Flex), hair dye (such as Lady Clairol) applied identifiers, non-toxic livestock markers (for example non-toxic, lead-free all-weather "paintstick" livestock markers by La-Co Industries), or shaving a small amount of hair on the top of the head. For example, pups that are captured in locations where they are likely to be resighted regularly may be flipper-tagged, whereas pups in remote locations lacking resight effort are often not flipper-tagged. Additionally, pups that are handled (weighed, measured, sampled, etc.) during capture operations, but not branded, are marked with paintsticks to identify individuals and prevent repetitive capture and sampling. Takes requested are a subset of the takes requested for captures under Activity 7. For example, during the 2003 season 25 tags were applied to pups that were below a minimum weight for branding.

i. Hot-brand. Pups to be branded are a sub-set of those pups <2 months old captured and measured on rookeries during the Steller sea lion breeding season (late June and early July; Tables 2; up to 800 per year from the eastern DPS). Small groups of pups (10 to 20 animals) are captured individually and held in nets and taken one-by-one to be weighed, measured, anesthetized with isoflurane gas, and branded. As stated above, typical anesthesia administration time is 8 to 12 minutes at a surgical plane, although under situations of slow induction and/or extensive sampling this can reach a maximum of 25 minutes. Branding irons are made of cold-rolled steel (approximately 10 mm stock); the dimensions of the largest digits are approximately 5cm wide and 8cm high (Merrick et al. 1996). Each iron is heated red-hot in a portable, propane-fired forge and applied perpendicularly to the animal's shoulder with light, even pressure (ca. 5 psi) for 2-4 seconds. Digits are 4-5cm apart to insure clarity of numbers. A 3-digit brand requires about 1-2 minutes to complete. Pups are observed during recovery from anesthesia, then released. Total handing time from hand capture to full recovery from anesthesia is typically 1 hour, however may extend to a maximum of 1.5 hours under conditions of unusually difficult terrain or slow recovery from the anesthetic that requires additional monitoring. Very young pups (e.g., under 20 kg or umbilicus present) are not branded in order to obtain a sample of post-natal, viable pups for survival analyses (see Appendix 1.). Sea lions 2 months to 3 years (100 in the eastern DPS and 150 in the western DPS) and 30 animals > 3 years of age (15 each in eastern and western DPS) will be branded under anesthesia following the same protocols (if not previously branded as pups). See Appendix 1 for a detailed analysis of branding activities. Only one brand is applied over the life of the animal, regardless of how well brands heal and readability of digits. Poorly healed (containing at least one unreadable digit) brands were few (<0.07 of animals resighted again at ≥ 1 yr of age) for animals branded as pups at rookeries 2001-2005 (ADFG unpublished data). A poorly healed brand also serves as a unique indentifier as photograph documentation prevents biasing of resight or survival probabilities by unreadability of digits.

j. Attachment of scientific instruments. Scientific instruments may be attached to up to 65 sea lions 2 months to 3 years old (up to 4 times per year; 10 in the eastern DPS and 55 in the western DPS) and to 30 adult sea lions ≥ 3 years of age (up to 2 times per year; 15 each in the eastern and western DPS; Table 2). Instruments are attached to the hair on the animal's back just over the shoulders, or onto the top of the head (if instrument and animal size are appropriate), with a small volume of fast-setting epoxy glue. All instruments attached this way will fall off during the annual summer-fall molt or are removed by investigators using shavers to clip the fur under the instrument at the time of subsequent capture, whichever come first. Attached instruments will allow tracking of sea lions at sea and measurement of behavior during dives (task 2.3.3, NMFS 2006). Instrument technology is developing rapidly as advances in miniaturization, battery technology and software engineering allow for new combinations of instruments. We will deploy instruments such as, but not limited to, time-depth recorders (TDR), satellite-linked dive recorders (SDR), satellite-linked time-depth recorders (SLTDR), and VHF transmitters that do not require instrument recovery to download data. We will also potentially deploy underwater cameras (UTPR), and TDR with physiological monitoring abilities (heart rate and/or stomach temperature telemeters), and perhaps acoustic recorders that require instrument retrieval if recapture of these individuals is planned. The maximum number of instruments attached to an individual sea lion will not exceed 2 to 5% of the animal's body weight (Kenward 1987, RIC 1998). This work will be conducted primarily in Prince William Sound, Alaska, but may occur range-wide in response to opportunities for collaboration with colleagues in other areas. The number of animals outfitted with instruments each year will be determined by budget limitations rather than more rigorous calculations of optimum or minimum sample sizes. Sufficient samples sizes for effective testing of hypotheses will be met through multi-year study designs.

k. Bioelectric impedance analysis. Bioelectric impedance analysis (BIA) is a rapid measure of body composition conducted on anesthetized animals. Up to 350 pups 5 days to 2 months in the eastern DPS, up to 250 juveniles ≥ 2 months to 3 years of age (up to 4 per year; 100 in the eastern and 150 in the western DPS) and up to 30 adults sea lions > estimated age of 3 years (up to 2 per year; 15 each in the eastern and western DPS) will be tested using this tool (Table 2). BIA measures the conductivity of a whole body as an index to the distribution of water and electrolytes, and uses measurements of reactance and resistance to calculate estimates of body fat (Lukaski 1987). This requires developing a mathematical relationship between values determined from BIA, and another measure of body composition, such as deuterated water dilution. This technique has been widely applied in marine mammals, with varying degrees of precision (Gales et al. 1994; Arnould 1995; Bowen et al. 1998; Bowen et al. 1999). Currently this technique looks very promising for assessing individual condition in Steller sea lions. A recent analysis by Castellini (2001) found an excellent relationship between deuterated water measures of total body water and BIA impedance measurements. Adding this determination to our methodology will expand the data used in creating the model. The procedure is simple and quick. On a sedated sea lion, four 1 ½ inch 20 G needles are inserted subcutaneously (two anterior just behind the skull, two posterior near the tail) as electrodes. Leads from these electrodes attach to a portable BIA unit (RJL Enterprises Quantum II, or Model 101A) and instantaneous readings of resistance and reactance are obtained. The electrodes are then removed. For best precision, the measures are repeated 2-5 times, taking a maximum of 3 minutes while animals are anesthetized . Although both techniques will be used on each animal simultaneously during the development phase of this new BIA technique, ultimately this BIA technique may replace deuterated water injection as a method to determine body composition, particularly in situations where waiting for the lengthy equilibration period is unfeasible, if the technique proves reliable in this species.

l. Deuterated water and NaBr. Deuterated water (D2O) and sodium bromide (NaBr) will be injected in up to 250 juveniles between the ages of 2 months to 3 years (up to 4 per year; 100 in the eastern DPS and 150 in the western DPS) and 30 adults > 3 years of age (up to 2 per year; 15 each in the eastern and western DPS) to determine total body water content and intracellular water space. Sea lions under gas anesthesia will be given an intramuscular injection of known volumes (1 g D2O/kg body mass) of deuterated water (99% enriched) using a 20 g 1.5" hypodermic needle after the injection site is cleaned with alcohol. These animals will also receive an intravenous injection of a known volume of NaBr (30-50 mg/kg body weight). A pre-sample of blood will be collected prior to injection of D2O and NaBr, and equilibration of the isotope will occur for approximately 2.5 hours while other procedures are being performed. A preliminary post sample of serum will be drawn 20 min prior to the final post sample, at approximately 2 hours. Separate post samples are advantageous for determining that full equilibration of D2O and NaBr have been accomplished. Methods and volumes of blood collected have been included and described under *7a. blood collection.* Mass spectrophotometric analysis of D2O in water distilled from blood samples will be conducted by a commercial laboratory (Metabolic Solutions, Inc.) or enrichment of samples will be measured using FTIR spectrophotometry. Calculation of percent body fat will be made using equations from Bowen and Iverson (1998), and assuming a hydration of 0.73 (Wang et al. 1999). NaBr concentration is determined using high pressure liquid chromatography (HPLC).

m. Evan's blue dye. The level of available oxygen stored in blood can be determined by measuring hematocrit and hemoglobin (routinely performed as part of the approved health condition blood sampling protocol), and total blood volume. Total blood volume will be estimated by injection and dilution of Evan's blue dye (ICDH 1973, Foldager and Blomqvist 1991). After collection of a pre-sample (same as the D2O pre-sample, above), Evan's blue dye will be injected intravenously at a dose of 3-5cc (0.5 mg/kg body weight), serial 5 ml blood samples will be collected at 10, 20 and 30 minutes post injection from up to 250 juveniles > 2 months to 3 years of age (up to 4 per year; 100 in the eastern DPS and 150 in the western DPS) and from up to 30 adults older than 3 years (up to 2 per year; 15 each in the eastern and western DPS). Evan's blue dye concentration is determined spectrophotometrically. Calculation of total blood oxygen storage capacity is made incorporating measures of blood volume, hematocrit, and hemoglobin concentration (Kooyman et al. 1980; Ponganis et al. 1993).

n. Stomach intubation. With up to 350 sea lions > 5 days and ≤ 2 months of age in the eastern DPS, 250 sea lions > 2 months but ≤ 3 years (up to 4 per year; 100 in the eastern DPS and 150 in the western DPS), and up to 30 sea lions > 3 years (up to 2 per year; 15 each in the eastern and western DPS; Table 2), we will collect a sub-sample of stomach contents using stomach intubation. A clean stomach tube (e.g. foal feeding tube) is inserted into the mouth and throat of anesthetized animals and gently guided down through the esophagus. A gentle suction will result in any stomach fluids wicking up the tube, which is then pinched, extracted, and the stomach contents drained into sample containers. Stomach tubes are cleaned with water and Nolvasan disinfectant and subsequently rinsed well with water before use on subsequent animals to avoid cross-contamination of samples and potential spread of disease between individuals. Samples are stored at -10C or on dry ice while at sea, shipped on dry ice and stored at -80C upon return to the laboratory until analysis. Analysis is completed in our ADFG/UAF laboratory or cooperating laboratories.

o. Determine metabolic rates by portable metabolic chamber. We plan to determine the whole animal metabolic rate of all sea lions captured during our physiology research (2 months to 3 years, 100 in the eastern DPS and 150 in the western DPS up to 4 times per year; all ages > 3 years, 15 in each eastern and western DPS up to 2 times per year) by indirect calorimetry using open circuit respirometry (a portable metabolic chamber). This technique has been used successfully in a captive setting both at the Vancouver Aquarium and at the ASLC, and most recently in field captures with our program to determine how much oxygen an animal consumes over time under various environmental and physiological conditions (Hoopes et al. 2005). The results of captive research have prompted authors to propose that a depressed metabolic state (lower relative oxygen utilization) would be evident in animals subject to nutritional stress. Metabolic rate is measured in animals while resting quietly in capture boxes during equilibration of deuterium for body composition measurements. This equilibration period provides an excellent opportunity to collect oxygen consumption data without increasing the holding time of the animals. This equilibration period follows initial blood sampling and thus is greater than one hour after initial capture of the juveniles such that any stress of capture has been reduced. During 2003-2005 we conducted this experiment on over 80 wild-caught animals with no noticeable effects. There is no evidence of stress caused by this restraint in the capture box (or metabolic chamber) either through visible observation or through changes in oxygen consumption. As in all other situations during our research, if there is ever a situation of an animal showing evidence of distress during capture, handling or restraint within the capture box, it will be at the discretion of the attending veterinarian to determine whether it is in the best interest of the animal to immediately release the animal from the restraint or to administer an anesthetic to relieve the stress.

The results of these metabolic rate measurements will be used in concert with other physiological indices of condition outlined above to test the hypothesis that animals in the areas of greatest decline are currently impacted by nutritional stress. Dr. Rea has experience in measuring metabolic rate in this manner in three pinniped species (hooded seals, elephant seals and California sea lions) and our collaborators from University of Central Florida (Dr. Graham Worthy and a Ph.D. candidate Lisa

Hoopes) have collectively been conducting this type of research for over 15 years.

The chamber is designed to completely enclose the occupied capture box, with multiple intake vents for fresh air entry at one end of the chamber and an exhaust port system through which the mixed ambient air and respired gases will be drawn by the vacuum pump provided in the Sable Systems metabolic rate analysis equipment. The flow rate of ambient air through this system is adjusted to ensure constant fresh air delivery, good mixing and negligible accumulation of expired gases. The chamber is constructed of transparent lexan to allow constant observation of the subject during the measurement. This ensures that we analyze data during rest, and not sleeping periods. Hoopes et al. 2005 found no significant difference in oxygen consumption when comparing metabolic rates during the period prior to initial anesthesia and then subsequently while animals were resting on deck between initial anesthesia and injection of deuterium, and the final anesthesia period during which the remainder of samples are collected.

In total, we will use this method on 250 Steller sea lions ages 2 months to 3 years (with a maximum of 4 takes per animal per year and a maximum of 2 takes per day for measurement of the effect of anesthesia events) and up to 30 animals > 3 years (up to 2 times per year). This will allow us to determine the resting metabolic rate of young sea lions at various times of the year to assess differences in metabolic status of animals between the eastern and western populations.

p. Ultrasonic imaging. We will use ultrasonography to measure blubber depth and to image internal organs of anesthetized sea lions (Table 2). Ultrasonography has become commonplace in wildlife and marine mammal medicine (Brook et al. 2001). On restrained sea lions, measurements of blubber depth will be obtained by directly imaging the blubber layer with a Sonosite 180 Plus with a general purpose curved transducer array used externally. If appropriately trained personnel are available, examination of internal organs (again with an external transducer) will be conducted. Up to 350 pups > 5 days to 2 months of age in the eastern DPS, 250 juveniles 2 months to 3 years (up to 4 times per year, 100 in the eastern DPS and 150 in the western DPS) and 30 adults >3 years (up to 2 times per year, 15 each in the eastern and western DPS) will be assessed.

<u>Expected Results</u> These data will provide information on the relative health of the population when compared to results from preceding years and among areas. Data obtained from deployed instruments will contribute to ongoing investigations into seasonal movements, diving behavior, habitat selection, and foraging ecology. This work will be particularly important for identifying winter foraging areas and refining our knowledge of the foraging capabilities of young sea lions. This information will be crucial in assessing the potential effects of commercial fisheries on the status of Steller sea lions. Resighting of hot-branded animals over time will provide critical information on survival and reproductive success.

3. Removing a Marine Mammal from the Wild: Not applicable.

4. Lethal Take: No intentional lethal takes are requested. Unintentional mortality of Steller sea lions is possible, particularly during capture and handling operations. During five years of similar research under Permit No. 358-1564, 14 mortalities were recorded during the capture and handling of 1200 sea lions. The only juvenile mortality occurring on ADFG permit since 2001 was a female sea lion that aspirated under anesthesia during a capture trip in April, 2004. Apart from that mortality

ADFG has captured over 650 sea lions using the underwater noose with perfect survivorship. No mortalities are expected as a result of pen captures, though post-capture anesthesia always has a potential for causing accidental mortality. During the past ten years, researchers at NMML have captured 1800 sea lions (predominantly California sea lions) in floating pens at Shilshole Bay, Washington with only a single mortality (Pat Gearin, pers. comm., reported under Permit #835 and 782-1446). Capture of sub-adult and adult sea lions using injectable drugs administered via remotely fired darts may result in mortalities. During 1990-1996, 1 of 53 (1.9%) sub-adult and adult sea lions that remained observable (that is, did not enter the water) following darting by NMML died. Because research-related mortalities are rare events compared to the number of sea lions taken from all activities, a calculated average rate may not be reached over several years, or may be exceeded within one year. Thus, we request accidental mortality takes of 10 (not to exceed 5 in the western stock) per year as authorized in our previous permit.

5. Import/Export of Marine Mammals/Marine Mammal Parts: Any importation of Steller sea lion parts or samples will be accomplished under the authority and conditions of existing ADFG permit No. 358-1564-7 and its successors.

D. Research Effects and Mitigation Measures

Small adverse impacts may result from harassment associated with aerial and brand resighting surveys, tagging, branding, and due to handling. Using protocols developed since the 1970's by NMFS, ADFG, and consulting veterinarians, the actual adverse impact should be negligible. A recent review of anticipated effects of these activities was presented in the environmental assessment for permitting research activities in 2002 (NMFS 2002).

1. Effects

Effects on Individual Animals:

For Activities 1-3, it is not possible to reliably estimate numbers of takes during these activities by age-class or sex and so we request number of takes for all age-classes/sexes combined.

Activity 1: aerial surveys during the non-breeding season at Alsek and Akwe River mouths
Survey aircraft approach sites, when possible, from a kilometer or more offshore and without banking (the sound change associated with banking increases the likelihood of disturbing animals), and they typically are within hearing range for no more than 1-2 minutes.

The following examples are taken from and analysis performed by NMML researchers:
"For example, the NMML final report for 782-1532 (for the years 2000-2004) reported 2,797 disturbed sea lions (similar to 'alerted') out of 216,821 counted during monthly aerial surveys in both western and eastern stocks, a rate of 0.013 sea lions alerted/counted. An objective of aerial surveys is to not disturb sea lions as that could negatively bias the sample if animals left the field of view. Observations from counters indicate it was very rare for seals to actually spook or go into the water. The NMML final report for 782-1532 also reported that <10% of sea lions counted during breeding season aerial surveys reacted, and that few animals spooked off of a site. Observers at field camps in 2002 and 2004 observed little response to survey aircraft, but reported "mild spooks" at Ugamak Island though all

animals remained on the beach. “Mild spooks” refers to a proportion of the animals (~10%) becoming alert and moving toward the water, but remaining on the beach. Reactions of animals to aerial survey aircraft differ depending on the acoustics of the site. A reaction similar to that observed at Ugamak Island is more likely at rookeries or haul-outs located at the base of a cliff or in an embayment. Little or no reaction of animals has been observed at sites on flat offshore islands.

Given the range of alert response rates with no age-class specificity (0.013 - <0.10), 0.05 was selected as an approximation for pups and non-pups. Since no pups were observed to enter water in response to aerial surveys, their enter water value was set to 0.0, and for non-pups to 0.01 entering water/counted (likely an overestimate based on field camp reports and the proportion of sites on flat offshore islands). Proportions of 0.001 (1/1000) for pups and 0.0001 (1/10,000) for non-pups as estimated rates of injury per number of individuals potentially exposed were based on NMML professional opinion. Pups were assumed to be more at risk than non-pups.”

Activity 2: Brand-resighting and associated observations

During resighting activities by skiff, disturbance to animals is minimized by slowly approaching the haul-out and backing away from the site when movement of animals due to our presence is observed, as described in Section C, Methods, Description of Activity 2. Commonly animals disturbed into the water haul-out again within 30 minutes of the disturbance. Particularly few animals are disturbed during resight projects (2)-(3), as natural, undisturbed behavior is required to collect data concerning reproductive and weaning probabilities.

The following examples are taken from and analysis performed by NMML researchers:

“Incidental effects account for the effects on the proportion of animals incidentally exposed to a research activity. Researcher presence among animals is expected to have different impacts depending on timing of the activity relative to the sea lion breeding cycle. Expected reactions of exposed sea lions include: 1) becoming alerted (includes physiological reactions that may not be externally expressed); 2) entering water; or 3) sustaining an injury because of the activity (for example, being trampled, or having an elevated physiological stress reaction).

Vessel surveys-proportion reacting

Because most vessel survey activities are pursuant to observing marked sea lions, it was assumed that all potentially exposed sea lions are alerted by this activity. Also, most researchers request takes for incidental disturbance as the number that are likely to be affected, rather than as the number that will be potentially exposed (as for aerial surveys). This is reflected in the proportion alerted of 1.0 alerted per potentially exposed sea lion. Proportions of sea lions entering the water (0.3 for pups and non-pups) were based on the proportions of animals entering the water during surveys for marked animals in the Gulf of Alaska and Aleutian Islands in 2004-2006. Proportions of 0.0001 (1/10,000) for both pups and non-pups as estimated rates of injury per number of individuals potentially exposed were based on NMML professional opinion, and are the same rates estimated for aerial surveys. Pups were assumed to be more at risk than non-pups.”

Activity 3: incidental disturbance during scat collection and capture/sampling activities

Scat collection occurs on haulouts and adults and juveniles are moved slowly as people approach from the water or land. When collecting scats or capturing/sampling animals, sea lions return to the haulout within minutes after departure of the scientific party. At some sites, animals have hauled-out along the periphery of the beach before the scientific party departed. When possible during in-water capture activities, people do not step onto the rocks and thereby reduce the disturbance.

The following examples are taken from and analysis performed by NMML researchers:
"Incidental effects account for the effects on the proportion of animals incidentally exposed to a research activity. Researcher presence among animals is expected to have different impacts depending on timing of the activity relative to the sea lion breeding cycle. Expected reactions of exposed sea lions include: 1) becoming alerted (includes physiological reactions that may not be externally expressed); 2) entering water; or 3) sustaining an injury because of the activity (for example, being trampled, or having an elevated physiological stress reaction).

On rookeries during breeding season-proportion reacting
Because these activities occur among animals on haulouts or rookeries, and most researchers request takes for incidental disturbance as the number that are likely to be affected, it is assumed that all sea lions listed as potentially exposed are at least alerted by this activity. This is reflected in the proportion alerted of 1.0 alerted per potentially exposed sea lion for all activities. Proportions of sea lions estimated to enter the water are based on NMML professional experience. Very few pups (estimated at 1/100) enter the water during rookery operations, but it is more common for non-pups (0.9) to do so during presence among animals on rookeries in the breeding season. Estimated rates of injury are the same as for incidental-in view disturbances. A sub-activity on rookeries involves moving pups into groups (roundups) for efficient handling during permanent marking (branding) or for collecting measurements and samples. Numbers listed under potentially exposed are the number of pups requested to be handled for those activities, and thus all "react" to the activity (proportion reacting = 1.0).
On haulouts (any season) and rookeries (non-breeding season)-proportion reacting
Because these activities are among animals on haulouts or rookeries, and most researchers request takes for incidental disturbance as the number that are likely to be affected, it was assumed that all sea lions listed as potentially exposed are at least alerted by this activity. This is reflected in the proportion alerted of 1.0 alerted per potentially exposed sea lion for all activities. Rates of pups or non-pups entering the water are assumed to be the same, since older pups will be more similar to non-pups in movement ability."

Activity 4: Collect carcasses. Carcasses are most often found alone, away from other animals such that no animals would be disturbed. A carcass that is within a group of animals will be collected if it is its freshness can be determined without disturbance to animals, it is fresh and particularly if the number of animals requiring disturbance for the collection is < 100 (such as a small haul-outs and in areas on the periphery of haul-outs). Older carcasses deemed valuable by veterinarians (age-classes or geographic areas of need to health/pathology studies) will be collected at sites >100 animals. Carcasses will not be collected in areas where there is the potential for pups < 2 mo of age to the disturbed.

Activity 5: Receive tissue samples from subsistence harvested sea lions. Not Applicable.

Activity 6: Incidental Mortality. Not Applicable

Activity 7: Capture by all methods listed and morphometrics: As of 2006, the ADFG program has captured over 650 sea lions using the dive-capture method with <u>no</u> mortalities directly associated with the capture technique. Approximately 3-10 juveniles have been captured on land each year since 2001 and the only juvenile mortality occurred during capture in May of 2004 (reported under permit 358-1564). Significant modifications have been made to pup capture and handling methods on Southeast Alaska rookeries during branding operations, following an episode of multiple pup deaths caused by asphyxia due to pup crowding and drowning in a pool (reported under permit #358-1564). This reflects the significant effort in modifying capture protocols to minimize crowding and asphyxia related mortalities. Although pups may struggle initially after capture, they typically calmed down very quickly after being hand-restrained. There were no instances of distress reactions (open mouth breathing, catatonia, etc.) to these activities. Much of our handling technique is designed to encourage pups to be calm while holding them firmly. In 2004 new protocols were developed for pup branding in Southeast Alaska that significantly reduced the risk of mortality from pups trampling each other while being captured. Using these improved protocols, approximately 400 pups were captured in June-July 2005 with only one anesthesia related pup mortality (reported under MMPA permit 358-1769). These methods will continue to be modified as appropriate for the terrain of individual rookeries. The following excerpt from our 5-Year Report of activities for Permit # 358-1564 describes in detail changes made to our rookery branding operations:

"During this permit period we have also made several modifications to our branding operations in Southeast Alaska with the intention of minimizing disturbance to the individual and group. During our first year of branding protocols were adopted based on the experience of researchers who had tagged pups in the western stock and those who had experience with hot branding of California sea lion pups in California. These protocols were appropriate for relatively flat terrain found in these locations, but proved problematic in the Southeast Alaska locations due to the rugged terrain, high population of pups and ever present standing pools. During the early attempts at branding in Southeast Alaska several mortalities occurred due to pups being crushed among the huddle of pups that were corralled for branding, or were caught in the pools present on the rookeries, often due to a large number of pups crowding and holding them below the surface. Several modifications to the original protocols have been made over the 5 years of branding in Southeast Alaska to mitigate these problems and minimize the overall disturbance that our presence on a rookery imparts. The following excerpt from our recent 2005 branding activities report describes the current protocol that has been successful at eliminating pup mortality from crowding and drowning. "At each rookery site a small group of four or five biologists surveyed the rock from a skiff to plan the best way to approach a site and move pups for branding. Once a location was chosen, the adults were slowly moved off the rocks. It was possible to put a large number of the adult animals into the water by approaching from the skiff and causing the initial disturbance by making noise and attracting attention. This allowed us to control the rate that animals entered the water (*i.e.* how fast and how many animals move into the water), thereby permitting a slower movement of the sea lions. Moving the adults slowly allowed time for pups to move away from the water, reducing the number of pups that go into the water when people move onto the rock. Once pups had moved away from the water and a large stampede was unlikely, the first group of biologists moved onto the rock and an initial area was cleared of animals. These biologists would then set up equipment and make a general plan. The branding operation was set up in relative seclusion and the pup hold/release site was situated so as not to scare unbranded pups. If done correctly unbranded pups would remain relatively close but undisturbed until pup roundups were conducted. Once most of the equipment and general working area had been set up, captures of pups began. This was done by stalking groups of pups that were in safe areas and netting them with small hoop nets. In a single round up, 15-20 pups could be captured and then taken to the branding area. Corralling of pups was avoided at all times and fencing was only used to prevent pups from being pushed into cracks or pools. Otherwise animals not in nets were not corralled or contained in any way, and free to move. Pups in nets were secured and watched by two to six people at all times to insure the safety of the animals until branding." These methods have proven successful in locations such as those found in Southeast Alaska with rugged terrain and a large population of pups."

The following paragraph was written by the NMML permit proposal but is describes the ADFG methods also. "When noosed underwater, sea lions would attempt to swim away, and resisted being pulled toward the skiff. However, once in a capture box sea lions always stopped struggling, and would often fall asleep. Similarly, sea lions hoop netted on land would struggle against the hoop net, but relaxed once physically restrained within the restraint jacket or hoop net. Time of handling for sea lions captured and handled on land was of short duration at 0.2-1.1 hours. Elapsed time held for sea lions captured in water or on land, and taken to the research ship was longer, ranging between 3-13 hours. Holding time varied depending on how many sea lions were captured, and the time of day at which they were captured. Much of that time, however, was spent waiting in the capture box. Actual time of procedures, reflected by the elapsed time an animal was under gas anesthesia, ranged from 0.7-1.9 hours."

a-g. Blood, skin, blubber, muscle, fecal and swabs, tooth, vibrissae collection. Experienced personnel collect all samples from anesthetized animals. Criteria for determining whether someone is adequately trained will be based on demonstrated expertise, previous experience, and referrals of qualified colleagues. There is no noticeable effect when collecting while the sea lion is under gas anesthesia. All of these collections are the same as addressed previously. Recaptures of animals have indicated that no adverse effect has occurred to animals that have undergone the listed procedures. Blubber/muscle biopsy and tooth removal sites have healed, as have genetic tissue collection sites. Behaviorally, previously captured animals have chosen to interact with divers within hours to days of their first capture. The volume of blood collected ($\leq$1 ml blood per kg body mass) is based on the body weight and has been recommended by marine mammal veterinarians as a negligible amount.

To reduce the risk of infection, only clean, sterile disposable needles, scapels and punches will be used to obtain blood samples and a new needle/punch will be used for each blood or tissue collection. The area to be sampled will be thoroughly disinfected with ethyl alcohol or betadine prior to insertion of the needle, scalpel or punch. Sufficient pressure and/or dry gauze will be applied to the venipuncture site after removal of the needle to minimize the potential for hematoma formation in the surrounding tissues. This procedure will only be performed by/under the direct supervision of qualified and experienced personnel.

i. Hot branding. See Appendix 2 for an analysis of effects of this activity compiled by NMML and ADFG.

j. Attachment of scientific instruments. Instruments are externally attached to the fur using fast-setting epoxy glue. Instruments attached this way will fall off during the annual summer-fall molt, or will be removed by investigators using shavers to clip the fur under the instrument at the time of recapture, whichever comes first. Dimensions and exact types of instruments deployed upon sea lions over the 5-year duration of this permit will vary due to improvements in technology; dimensions of current technology we are most likely to employ are described in Table A.

Table A Sample externally-mounted instrument dimensions.

Instrument Type	Dimensions (length width height, cm)	Mass in air (g)
TDR	6 • 1.75 • 1.75	40
SPLASH (large)	10.6 • 3.5 • 3.5	145
SPLASH (small)	7 • 3 • 3	145
SRDL	10.5 • 7 • 4	370
SPOT	7.1 • 3.4 • 2.3	145
VHF	3 • 1 • 1	30
HTR	7 • 5 • 1	60
HRX	13 • 6 • 3.5	46

Preferred mounting location upon the animal depends on the type of instrument used. Head-mounting is preferred only for those instruments that perform best when they break the water surface between dives: dive recorders (time-depth recorder, TDR; satellite data recorder, SPLASH (formerly named SDR and SLTDR); satellite-relay data logger, SRDL) which use the water surface as a criterion for identifying dives, and radio transmitters (SPLASH, SRDL, SPOT, VHF) which can not transmit while submerged. Head mounting criteria require: instrument rests squarely upon the top of the head and leading edge of instrument rests behind a line connecting the most caudal portions of the eyes. Median dimensions of this area measured on wild captured sea lions (ADFG) are described in Table B.

Table B. Dimensions of sea lion head area meeting instrument head-mount protocol by capture age.

Age (months)	n	Length (cm)	Width (cm)
3	15	9	6
5	2	9.5	7.5
7	4	11	8
11	11	10	7
14	24	11	7
15	1	12	7
19	1	11	6
23	6	11	8
26	2	12.5	7.5
35	1	12	6

Dorsal mounting, along the midline over the shoulders, is adequate for archival instruments (heart-rate and stomach temperature recorder, HTR; heart-rate transmitter, HRX; archival camera, UTPR) and for mounting dive recorders or transmitters (TDR, SPLASH, SRDL, SPOT, VHF) upon smaller sea lions that do not meet head-mounting criteria. However, dorsal mounts are less likely than head mounts to break the water surface for long periods between dives, reducing radio transmission success and causing outlier dive durations due to missed dive termination events.

Deployment of more than one instrument upon an individual sea lion may be required, typically by combining transmitters with archival dive- or physiological parameter recorders (TDR, HTR, HRX, UTPR). Transmitters and archival tags may also be potted into single units. Multiple tags may be deployed as head+dorsal or dorsal+dorsal combinations; where head-mount is preferred for both instruments, combined dimensions will not exceed head-mount protocol. The maximum number of instruments attached to an individual sea lion will not exceed 2 to 5% of the animal's body weight (Kenward 1987, RIC 1998). Between 1998-2006, ADFG has successfully deployed TDR, SPLASH, SRDL, SPOT and VHF instruments upon free ranging <3 y old Steller sea lions. The majority of this work will be conducted in Prince William Sound, Alaska, but may occur range-wide in response to opportunities for collaboration with colleagues in other areas.

k. Bioelectric impedence analysis. This procedure involves an imperceptible electric current and is conducted on animals while under anesthesia.

l and m. Deuterated water and NaB and Evans Blue Dye. These solutions are injected while the sea lion is under anesthesia and then measured again in a subsequent blood draw. There are no known hazards to the individual sea lion.

n. Stomach intubation. Stomach intubation simply involves feeding a small foal feeding tube down the throat of an anesthetized sea lion and drawing out a couple ounces of contents. Sea lions have not shown a detectable response to this procedure in the past.

o. Metabolic chamber. As noted previously, this method involves setting the entire box containing a captured sea lion in an air tight lexan box and then measuring respired air. There is no detectable effect to the sea lion.

p. Ultrasonic imaging. This is a non invasive tool to examine internal structures and is performed on an anesthetized sea lion.

Effects on Stocks:
A review of anticipated effects of these activities was presented in the environmental assessment for permitting research activities in 2002 (NMFS 2002).

Activity 6: Accidental mortality
This Activity provides for accidental mortalities that may result from our research activities. During four years of similar research under Permit No. 358-1564, ADFG had 2 juvenile mortalities occur during a capture trip in 2004 and 15 pups die during branding operations. Assuming a NMML pup mortality rate and a pooled NMML/ADFG capture mortality rate (see data provided in application for permit #358-1769), an average of 1.4 mortalities per year (with a 1SE range of 0-2.8 mortalities per year) could be expected as a result of our proposed pup and juvenile capture and handling activities. No mortalities are expected as a result of pen captures, though post-capture anesthesia always has a potential for causing accidental mortality. During the past ten years, researchers at NMML have captured 1800 sea lions in floating pens at Shilshole Bay, Washington with only a single mortality (Pat Gearin, pers. comm., reported under Permit# 835 and 782-1446). The following information is provided by the NMML: Capture of sub-adult and adult sea lions using injectable drugs administered via remotely fired darts may result in mortalities. During 1990-1996, 1 of 53 (1.9%) sub-adult and adult sea lions that remained observable (that is, did not enter the water) following darting by NMML died. Because research-related mortalities are rare events compared to the number of sea lions taken from all activities, a calculated average rate may not be reached over several years, or may be exceeded

within one year.

In the Environmental Assessment prepared for issuing Steller sea lion research permits in 2002, NMFS (2002) established an average annual mortality upper limit that was applied to the western stock at a level that even if reached would not cause a significant impact. NMFS (2002) stated that if accidental mortalities in the western stock reached 10 sea lions in one year (about 5% of the stock's PBR) then researchers were required to consult with one another to identify research practices and prevent accidental mortalities in the western stock from exceeding 20 sea lions in that year (10% of the stock's PBR). With this mitigation measure in place, NMFS (2002) concluded that accidental mortality from research activities would not have a significant adverse impact on the Steller sea lion population.

Effects of Incidental Harassment:
Activity 1: aerial surveys during the non-breeding season at Alsek and Akwe River mouths. Harbor seals (< 300, all ages) near the mouths of the Alsek and Akwe Rivers may be incidentally disturbed during aerial surveys.

Activity 2: Brand-resighting and associated observations: Harbor seals (< 200/ year) and California sea lions (< 20/year) may be incidentally disturbed during resighting surveys, when hauled-out near sea lions. Northern fur seals (< 5 adult males, < 30 adult females and < 30 pups) at Hazy and Forrester Islands during brand resight work.

Activity 7: Captures. Harbor seals (< 50) and the occasional Northern fur seals (< 5 adult males, < 30 adult females and < 30 pups) on the Hazy Islands may be disturbed during sea lion pup branding.

Activity 8: Incidental disturbance during aerial surveys, scat collection, instrument retrieval, brand resight and capture/sampling activities on Steller sea lions. Covered above on Activities 1, 2, 7.

2. Measures to minimize effects

Minimize Stress, Pain, and Suffering:
A review of anticipated effects of these activities was presented in the environmental assessment for permitting research activities in 2002 (NMFS 2002). The state of Alaska Animal Use and Care committee has also reviewed and approved all proposed activities.
The capture and handling involved in Activity 7 are likely to elicit the greatest amount of stress during the actual capture operation. Methods and equipment are constantly being refined to limit the amount of stress and reduce any potential pain or suffering associated with capture. To date there are no other methods of capture known that would increase the safety or reduce the stress even more that what is currently used. Underwater captures have greatly reduced the potential for injury compared to land based captures and additionally have facilitated faster handling times. The majority of handling is conducted while the sea lion is immobilized under general anesthesia (isoflurane) and thus the stress, pain, and suffering are reduced as much as they are for human subjects undergoing much more invasive procedures.

Activity 7: Capture (includes hand, hoop net, underwater noose, floating trap, dart injection)
Capture myopathies have not been documented to occur in pinnipeds, though we assume captures are a

stressful event for sea lions. Captured animals are carefully monitored for signs of stress. If a captured animal shows signs of acute or protracted alarm reaction (e.g., overexertion, constant muscle tensions, abnormal respiration or heart rate) that may lead to serious injury, capture myopathy, or other disease conditions, or death, research-related procedures are immediately ceased to focus on the animal, and treat the symptoms as determined appropriate by the PI, CI, or attending veterinarian.

Animals are processed in groups small enough that all animals can be adequately monitored (*e.g.* two physically restrained but not chemically immobilized animals per observer), and handling/restraint times are kept to the minimum possible. When pups are handled, they are sufficiently monitored, and separated if necessary, to ensure that they are not suffocated, being crushed, or aspirating milk.

An experienced marine mammal veterinarian is present to carry out or provide direct on-site supervision of all activities involving the use of injectable and gas anesthesia.

Caution is exercised when approaching all pinnipeds, particularly mother/pup pairs, and efforts to approach and handle a particular animal or mother/pup pair are immediately terminated if there is any evidence that the activity(ies) may be life-threatening.

Reasonable steps will be taken to identify pups of lactating females before attempting to immobilize a lactating female. In the event a female dies or is seriously injured as a result of the activities, the orphaned pup shall, when it can be identified, be humanely provided for (i.e. salvaged [placed in a stranding facility for rehabilitation and eventual release], or if salvage is not possible, euthanized).

We ensure that animals that have been captured or are recovering from immobilizing drugs have an opportunity to recover without undue risk of injury from other animals by releasing them in an area void of other animals, yet in close proximity to the sampling stations where they can be monitored.

To the maximum extent practical without causing further disturbance of the rookery/haulout, animals are monitored post-handling for signs of acute stress or injury.

a. Blood collection

For this and other invasive procedures, only highly-experienced and well-trained personnel perform invasive procedures.

Not more than 1 ml of blood per kg body mass is drawn per capture event. When conducting labeled water and Evans blue studies, additional needle insertions may be performed but catheters are preferentially used to minimize impacts on the animal. If an animal cannot be adequately immobilized for blood sampling, efforts to collect blood are discontinued.

b. Muscle biopsy
c. Skin/ tissue biopsy
d. Blubber biopsy
k. Bioelectric impedance analysis
l. Deuterated water and NaBr
m. Evan's blue dye
n. Stomach intubation or enemas

Disposable needles, biopsy punches, etc., are used to the maximum extent possible (disposable needles are always used for blood sampling and injections of drugs or other approved substances).

When disposables are not available, instruments (darts, stomach tubes, biopsy needles, etc) are autoclaved or thoroughly disinfected with a bacteriocidal/virucidal agent in accordance with the product directions between animals and, as needed, immediately prior to each use.

f. *Tooth extraction*
Performed while sea lion is under general anesthesia, and only by trained personnel. Dental extraction instruments are thoroughly disinfected with a bacteriocidal/virucidal agent in accordance with the product directions between animals and, as needed, immediately prior to each use.

i. *Hot-brand* See complete discussion in Appendix 1.

Measures to Minimize Disturbance:
All activities are coordinated with research activities of National Marine Mammal Lab, Alaska SeaLife Center, UAF, Aleutians East Borough, University of Washington, Texas A&M and other researchers to minimize disturbance.

The Alaska Region is notified in advance of our research activity schedule.

Activity 1: Aerial surveys near Alsek river mouth. Survey aircraft approach sites, when possible, from a kilometer or more offshore and without banking (the sound change associated with banking increases the likelihood of disturbing animals), and they typically are within hearing range for no more than 1-2 minutes.

Activity 2: Brand-resighting and associated observations. Disturbance to animals is minimized by slowly approaching the haulout from up wind, allowing animals to become accustomed to our presence. If animals begin to move due to our presence, we back away from the site.

Activities 3-4: moving animals to collect scat, carcasses. Impacts of the these activities are potentially greater than from aerial surveys or brand-resighting because most adult animals must be moved to the edge of the rookery or into the water to land on the haulout to collect scat and in some situations, so collect carcasses. To minimize impact, our protocol includes the following:

a) Scat is not collected from rookeries during the pupping season unless the rookery is already disturbed for pup branding and then only in the area already disturbed. That is, no additional animals are disturbed. Haulouts are cleared of animals slowly by approaching from up wind and slowly moving the animals off the rock or further down the rock. This allows animals to slowly move to the water without stampeding.

b) minimize the time that we are occupying the beach

c) use biologists experienced in herding to slowly move the adults out of the way, and experienced counters to complete the surveys as quickly as possible.

The criteria used to determine when disturbance occurs are based on observations of animals reacting to the scientists. If animals are observed to be unable to move or are endangering themselves in response to scientists' activities, the activity is terminated. In the past we have terminated the approach to a haulout if it was observed that pups could be injured or fall into cracks. There are no quantifiable criteria to be used because each site has different topography and conditions and those are modified by the density of animals and distribution of sex/age classes.

In order to minimize disturbance to individual haulout sites, we have designed capture methods (underwater dive capture) and observation methods (remote cameras and observing from blinds) to minimize and when possible eliminate disturbance. In addition, when handling animals or moving animals, biologists experienced in capture and sampling techniques are used to complete the activities as quickly as possible.

i. Hot-brand

To minimize disturbance related to branding activities, we follow clearing procedures as described in the attached appendix. The portion of the rookery cleared is kept to the minimum necessary to round up the desired number of pups. The area used for the actual procedure is located out of sight of the majority of the rookery whenever possible in order to reduce the visibility. Likewise, pups are released out of sight after the procedures and following a recovery to ambulatory state. A minimum of one and usually three or more marine mammal veterinarians are on site to examine pups and administer care as they feel necessary. Emergency drugs and resuscitation materials are kept available and stocked and all personnel are trained to look for signs of distress in both pups handled or about to be handled and those possibly disturbed by our actions. These are also kept available for captures and handling of juveniles and animals > 3 years of age.

3. Monitoring effects of activities

All animals are observed closely after gas anesthesia to ensure full recovery from restraint prior to release. Animals are held in a protected location on the rookery or on the back of the research vessel during recovery under the protection of researchers until they regain mobility and move away under their own power. During capture events, significant effort is expended in conducting multiple resight bouts at haulouts to document behavior of recently handled animals and all branded animals observed. Over 50 animals have now been recaptured following previous branding and sampling events and all invasive procedures have been noted to be healed or healing in the expected manner (depending upon the length of time between captures) by veterinary staff.

4. Alternatives

All activities and protocols proposed have been previously reviewed and approved by the ADFG ACUC and a copy of our signed approval will be delivered with the hard copy of this application. Through consultation with leading marine mammal veterinarians, the least intrusive methods available for each activity have been selected to gain the information needed. All intrusive activities are conducted under chemical restraint.

E. Resources needed:
The most recent research proposal sent to the NMFS funding office is included with this package as Appendix 2.

Sponsors and Cooperating Institutions

1. National Marine Mammal Laboratory
Alaska Fisheries Science Center
National Marine Fisheries Service, NOAA
7600 Sand Point Way NE
Seattle, Washington

2. Protected Resources Management Division
Alaska Region
National Marine Fisheries Service
Juneau, Alaska

3. Alaska Maritime National Wildlife Refuge
U.S. Fish and Wildlife Service
2355 Kachemak Bay Drive, Suite 101
Homer, Alaska

4.Alaska Fish and Wildlife Research Center
U.S. Fish and Wildlife Service
1011 E. Tudor Rd.
Anchorage, Alaska

5. Institute of Marine Science
University of Alaska, Fairbanks
Fairbanks, Alaska

6. Marine Advisory Program
University of Alaska Sea Grant
Kodiak, Alaska

7. Alaska Sea Life Center
Seward, Alaska

8. Department of Marine Biology
Texas A&M University
Galveston, Texas

9. Marine Mammal Section
Fisheries and Oceans Canada
Nanaimo, British Columbia,

10. Department of Zoology
University of British Columbia
Vancouver, British Columbia

11. National Marine Fisheries Service
Southwest Fisheries Science Center
La Jolla, CA

12. Tongass National Forest
United States Forest Service
Yakutat Ranger District
Yakutat, AK

13. Glacier Bay National Park
National Park Service
Gustavus, AK

In addition, samples collected from Steller sea lions have been and will continue to be shared with researchers from institutions and agencies not listed here. These recipients will be included in the annual permit report as in the past.

Coordination between research teams from other agencies is accomplished through annual coordination meetings, through annual notification of research intentions to the NMFS Research coordinator, and through participation on scientific review panels. For example, in addition to frequent communications and meetings with NMML, ASLC and NPUMMRC personnel, Lorrie Rea also serves on the Scientific Review Committee for the NPUMMRC annual research and proposal review and holds a seat on the ASLC Scientific Advisory Committee. In addition to coordination of research, a very high level of collaborative research (sample sharing, joint captive projects etc.) have been undertaken with ASLC, NPUMMRC and NMML, which have also resulted in joint publication of results.

F. Publication of Results

Results will be presented in peer-reviewed publications, as presentations at scientific and management meetings or conferences, and in reports and presentations prepared for MMPA, ESA or NEPA analyses of proposed federal actions.

V. National Environmental Policy Act (NEPA) Considerations:

1. ADFG continues to develop the underwater capture technique for juvenile Steller sea lions and continually shares any upgrades to our protocols with researchers from other agencies. In some cases this exchange of information is accomplished through joint research cruises. For example during the past 2 years we have conducted joint captures with ASLC in Prince William Sound and with NMML in the Aleutian Islands. We will also work closely with NMML in the coming years to assist in the development of the at-sea net capture technique for Steller sea lions as appropriate. NMML's proposed project will involve the use of a new capture technique, which was presented by Simon Goldsworthy of La Trobe University, Victoria, Australia, at the 2004 Sea Lions of the World Conference in Anchorage,

Alaska. The technique has been successful for S. Goldsworthy and colleagues and we believe the method can be used to capture larger, older Steller sea lions in remote areas of Alaska. NMML will lead in the testing of this technique, and if successful, this technique will surely be adopted by other researchers in the future.

2. All personnel that handle biological samples have been instructed in the safe handling and storage of potentially infectious substances, however, we are unaware of any potential public health or safety concerns that may arise as a result of our proposed research activities. We also have dedicated members of our team who have been specifically trained in the handling of hazardous materials for shipping purposes.

3. Additional permitting is required for research within the Alaska Maritime National Wildlife Refuge and the Glacier Bay National Park, but the activity is not known to be a violation of any Federal, State, or local law or impact the physical environment to an extent that it is not in compliance with the protections for that area.

4. No loss or destruction of significant scientific, cultural, or historic resources is known to occur.

5. There is no known transportation of biological material from one area to another. All tissues collected during the course of research are stored dry or frozen onboard the vessel where there is no chance of exposure to subsequently captured animals or locations visited.

General: All techniques used in this study are currently used in wildlife biology/medicine studies and the majority of work described here is also conducted by the NMML and the Alaska Sea Life Center. In fact the Steller Sea Lion Recovery Team has recommended that different research groups collaborate and share activities and techniques. In past environmental assessments, activities related to hot-iron branding have been considered controversial. Please see Appendix 2 for a review of this methodology. The only "new" activities included in this application which have not already been approved under permit No. 358-1564 and its amendments are the use of Sodium Bromide in Activity 7.l, and the use of a portable metabolic chamber.
Northern fur seals breed on the Hazy Islands in Southeast Alaska, the site of pup branding on alternate years. Because the proposed activities have been used in Steller sea lions for at least the last two years (deuterated water, evans blue, muscle biopsy) through decades (aerial surveys, ground counts, instrumenting, branding), the potential effects are relatively well understood. By coordinating research with other research programs the cumulative effects are minimized. With measures in place for minimizing disturbance and a mortality limit, there does not appear to be a likelihood of adverse impacts to Steller sea lion stocks.

VI. Previous and other Permits.
Currently the Steller sea lion research program at ADFG is authorized under Marine Mammal Permit No. 358-1769 which was limited by federal court action on May 26, 2006 and subsequently by amended court action on June 30, 2006.
Importation/exportation of tissues or samples will be carried out under the authority of the NMML's NMFS Permit # 782-1399.
ADFG operates within the Alaska Maritime National Wildlife Refuge in the Gulf of Alaska and Aleutian Islands under the authority of a Special Use Permits from the Alaska Maritime National

Wildlife Refuge, Homer, Alaska. ADFG has also requested an annual permit from Glacier Bay National Park to conduct resights and brand pups.

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VI. 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 the following statutes and the regulations promulgated there under, as indicated in 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

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

The Fur Seal Act of 1966 916 U.S.C. 1151-1175).

I also understand that any false statement may subject me to the 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".

___________________________________ __________________

Matt Robus, Director date
Division of Wildlife Conservation
Alaska Department of Fish and Game

Table 1. Activities to be performed by each Principle and Co-investigator.

Co-investigator name	Title	Activities to be performed without supervision
Lorrie Rea	PI, Wildlife Physiologist III, ADFG	all except anesthesia
Kelly Hastings	CI, Wildlife Biologist III, ADFG	1-7, and 7a,c,d,e,g,h,j,k,l,n,p
Carrie Beck Eischens	CI, Wildlife Biologist III, ADFG	2-7, and 7a,b,c,d,e,g,h,j,k,l,m,n,p
Lauri Jemison	CI, Wildlife Biologist II, ADFG	1-7, and 7a,c,d,e,g,h,j,k,l,n,p
Ken Pitcher	CI, Wildlife Biologist III, ADFG	all except anesthesia
Mike Rehberg	CI, Wildlife Biologist II, ADFG	2-7, and 7a,c,g,h,i,j,k,l,n,p
Cheryl Clark	CI, Wildlife Biologist II, ADFG	1-7, and 7a,b,c,d,e,g,h,j,k,l,m,n,p
Jamie King	CI, Wildlife Biologist I, ADFG	1-7, and 7a,b,c,d,e,f,g,h,i,j,k,l,n,p
Greg Snedgen	CI, Boat Officer II, ADFG	1-7, and 7c,f,g,h,i,j,k,n,p
Dennis McAllister	CI, Fish and Wildlife Tech V, ADFG	1-7, and 7c,g,h,i,n
Grey Pendleton	CI, Biometrician, ADFG	1-7, and 7c,g,h,n
Kimberlee Beckmen (D.V.M.)	CI, Wildlife Veterinarian, ADFG	all, including anesthesia
Kathy Burek (D.V.M.)	CI, Veterinary Pathologist, AVPS	all, including anesthesia
Patricia Rivera	CI, Laboratory Technician, UAF	2-7, and 7a,b,c,d,e,g,h,k,l,m,n,o,p
Bob Small	CI, Wildlife Scientist I, ADFG	1-7, and 7c,f,g,h,i,j,k,n,p
Kim Raum-Suryan	CI, Wildlife Biologist, Sea Gypsy	1-7, and 7a,c,d,e,g,h,j,k,l,n,p
Shawn Johnson (D.V.M.)	CI, Wildlife Veterinarian	all, including anesthesia
Elizabeth Van Burgh	CI, Fish and Wildlife Technician, ADFG	1-7, and 7c,f,g,h,i,k,n,p
Steve Lewis	CI, Wildlife Biologist I, ADFG	1-7, and 7c,f,g,h,i,k,n,p
Tom Gage	CI, Fish and Wildlife Technician, ADFG	all excluding anesthesia, branding
Sue Goodglick	CI, Fish and Wildlife Technician, ADFG	2-7
Molly Kemp	CI, Fish and Wildlife Technician, ADFG	2-7
Nick Olmsted	CI, Fish and Wildlife Technician, ADFG	2-7
Jamie Womble	CI, Biologist, GBNP	1-7, and 7c,f,g,h,j,k,n,p
Karin Harris	CI, Biologist, GBNP	1-7
Karen Blejwas	CI, Wildlife Biologist II, ADFG	1-7, and 7c,f,g,h,k,n,p
Jo-Ann Mellish	CI, Assistant Research Professor, UAF	7 and 7a,b,c,d,e,g,h,j,k,l,m,n,p
Lisa Hoopes	CI, PhD. Candidate, UCF	7 and 7o

Susan Oehlers	CI, Biologist, National Forest Service	1 (aerial survey only)
Nate Catterson	CI, Biologist, National Forest Service	1(aerial survey only)
Thomas Gelatt	CI, Wildlife Biologist, NMML	all except anesthesia
Brian Fadely	CI, Wildlife Biologist, NMML	all except anesthesia
Julie Richmond	CI, Ph.D. candidate, U Conneticut	7 and 7a,b,c,d,e,g,h,k,l,m,n,p
Vicki Stegall	CI, Ph.D. candidate, UAF	7 and 7a,b,c,d,e,g,h,k,l,m,n,p
Don Calkins	CI, SSL program manager, ASLC	2-7, 7a,c,g,h,n
Andrew Trites	CI, MMRU program director, UBC	2-7
William Taylor (D.V.M.)	CI, Wildlife Veterinarian	all, including anesthesia
Mille Gray	CI, Veterinary Technician, ASLC	all, including anesthesia
Frances Gulland (D.V.M.)	CI, Wildlife Veterinarian, Director MMC	all, including anesthesia
Vicki Vanek (D.V.M.)	CI, Wildlife Biologist, ADFG	all, excluding anesthesia and branding
Pam Tuomi (D.V.M.)	CI, Wildlife Veterinarian, ASLC	all, including anesthesia

Table 2. Steller sea lion research takes requested by the Alaska Dept. of Fish and Game Steller Sea Lion Research Program 2007-2012

Species	Life Stage	Sex	Take Action (Activity)	# Animals taken/year	Maximum # Takes/ animal/ year	Season	Location
Eumetopias jubatus	> 9 months old	Both	1. Aerial surveys photograph non-pups during congregation on Alsek and Akwe River mouths	20,000	10	Feb - May annually	Alsek and Akwe River mouths, Southeast AK , Eastern DPS
Eumetopias jubatus	all ages	Both	2. Incidental disturbance during brand-resight activities	24,500 500	3 20	all year all year	Eastern DPS Forrester Island Complex, Eastern DPS
Eumetopias jubatus	all ages	Both	2. Incidental disturbance during brand-resight activities	5,000	3	all year	Western DPS
Eumetopias jubatus	all ages	Both	3. Incidental disturbance during ground counts, scat collection, capture/sampling activities and instrument retrieval	15,000	3	all year	Eastern DPS
Eumetopias jubatus	all ages	Both	3. Incidental disturbance during capture/sampling activities, and instrument retrieval	2,000	4	all year	Western DPS
Eumetopias jubatus	all ages	Both	4. Collect carcasses or parts of carcasses of Steller sea lions	unlimited	1	all year	Alaska-wide
Eumetopias jubatus	all ages	Both	5. Receive tissue samples [hard/soft parts] from subsistence harvested sea lions.	unlimited	1	all year	Alaska-wide

Eumetopias jubatus	all ages	Both	6. Incidental mortality	10 (not to exceed 5 in western stock)	1	all year	Eastern & Western DPS
Eumetopias jubatus	newborn to 2 months > 2 months to 3 years > 2 months to 3 years all ages > 3 years all ages > 3 years	Both Both Both Both Both	7. Capture (includes hoop net, noose, underwater lasso, floating trap, darting with Telazol, at-sea net) and Restraint (restraining net, Valium, isoflurane) and morphometrics	800 100 150 15 15	1 4 4 2 2	June-July all year all year all year all year	Eastern DPS Eastern DPS Western DPS Eastern DPS Western DPS
Eumetopias jubatus	> 5 days to 2 months > 2 months to 3 years > 2 months to 3 years all ages > 3 years all ages > 3 years	Both Both Both Both Both	7.a. Blood collection ≤ 1 ml blood per kg body mass per capture event.	350 100 150 15 15	1 4 4 2 2	June-July all year all year all year all year	Eastern DPS Eastern DPS Western DPS Eastern DPS Western DPS
Eumetopias jubatus	> 2 months to 3 years > 2 months to 3 years all ages > 3 years all ages > 3 years	Both Both Both Both	7.b. Muscle biopsy at time/ site of blubber biopsy	100 150 15 15	2 2 2 2	all year all year all year all year	Eastern DPS Western DPS Eastern DPS Western DPS
Eumetopias jubatus	> 5 days to 2 months > 2 months to 3 years > 2 months to 3 years all ages > 3 years	Both Both Both Both Both	7.c. Skin biopsy	800 100 150 15 15	1 2 2 2 2	June-July all year all year all year all year	Eastern DPS Eastern DPS Western DPS Eastern DPS Western DPS

	all ages > 3 years						
Eumetopias jubatus	> 5 days to 2 months	Both	7.d. Blubber biopsy	20	1	June-July	Eastern DPS
	> 2 months to 3 years	Both		100	4	all year	Eastern DPS
	> 2 months to 3 years	Both		150	4	all year	Western DPS
	all ages > 3 years	Both		15	2	all year	Eastern DPS
	all ages > 3 years	Both		15	2	all year	Western DPS
Eumetopias jubatus	> 5 days to 2 months	Both	7.e. Fecal loops and culture swabs	350	1	June-July	Eastern DPS
	> 2 months to 3 years	Both		100	4	all year	Eastern DPS
	> 2 months to 3 years	Both		150	4	all year	Western DPS
	all ages > 3 years	Both		15	2	all year	Eastern DPS
	all ages > 3 years	Both		15	2	all year	Western DPS
Eumetopias jubatus	> 2 months to 3 years	Both	7.f. Tooth extraction (only 1 tooth is taken over the life of an animal)	30	1	all year	Eastern DPS
	> 2 months to 3 years	Both		30	1	all year	Western DPS
	all ages > 3 years	Both		15	1	all year	Eastern DPS
	all ages > 3 years	Both		15	1	all year	Western DPS
Eumetopias jubatus	> 5 days to 2 months	Both	7.g Pull vibrissae, clip hair and nails	20	1	June-July	Eastern DPS
	> 2 months to 3 years	Both		100	2	all year	Eastern DPS
	> 2 months to 3 years	Both		150	2	all year	Western DPS
	all ages > 3 years	Both		15	2	all year	Eastern DPS
	all ages > 3 years	Both		15	2	all year	Western DPS

Eumetopias jubatus	newborn to 2 months	Both	7.h. Flipper tag / tissue sample	200	1	June-July	Eastern DPS
	> 2 months to 3	Both	(may retain skin punch for genetic	100	2	all year	Eastern DPS
	years	Both	analysis)	150	2	all year	Western DPS
	> 2 months to 3	Both		15	2	all year	Eastern DPS
	years	Both		15	2	all year	Western DPS
	all ages > 3 years						
	all ages > 3 years						
Eumetopias jubatus	> 5 days to 2	Both	7.i. Hot-brand	800	1	June-July	Eastern DPS
	months	Both	(only one brand over life of animal)	100	1	all year	Eastern DPS
	2 months to 3 years	Both		150	1	all year	Western DPS
	2 months to 3 years	Both		15	1	all year	Eastern DPS
	all ages > 3 years	Both		15	1	all year	Western DPS
	all ages > 3 years						
Eumetopias jubatus	≥ 2 months to 3 yrs	Both	7.j Attachment of scientific instruments	10	4	all year	Eastern DPS
	≥ 2 months to 3 yrs	Both		55	4	all year	Western DPS
	all ages >3 years	Both		15	2	all year	Eastern DPS
	all ages >3 years	Both		15	2	all year	Western DPS
Eumetopias jubatus	> 5 days to 2	Both	7.k. Bioelectric impedance analysis	350	1	June-July	Eastern DPS
	months	Both		100	4	all year	Eastern DPS
	2 months to 3 years	Both		150	4	all year	Western DPS
	2 months to 3 years	Both		15	2	all year	Eastern DPS
	all ages > 3 years	Both		15	2	all year	Western DPS
	all ages > 3 years						
Eumetopias jubatus	2 months to 3 years	Both	7.l. Deuterated water and NaBr (sodium	100	4	all year	Eastern DPS
	2 months to 3 years	Both	bromide)	150	4	all year	Western DPS
	all ages > 3 years	Both		15	2	all year	Eastern DPS
	all ages > 3 years	Both		15	2	all year	Western DPS
Eumetopias jubatus	2 months to 3 years	Both	7.m. Evans blue dye	100	4	all year	Eastern DPS
	2 months to 3 years	Both		150	4	all year	Western DPS
	all ages > 3 years	Both		15	2	all year	Eastern DPS
	all ages > 3 years	Both		15	2	all year	Western DPS

Eumetopias jubatus	> 5 days to 2	Both	7.n. Stomach intubation	350	1	June-July	Eastern DPS
	months	Both		100	4	all year	Eastern DPS
	2 months to 3 years	Both		150	4	all year	Western DPS
	2 months to 3 years	Both		15	2	all year	Eastern DPS
	all ages > 3 years	Both		15	2	all year	Western DPS
	all ages > 3 years						
Eumetopias jubatus	2 months to 3 years	Both	7.o. Determine metabolic rates by portable	100	4	all year	Eastern DPS
	2 months to 3 years	Both	metabolic chamber	150	4	all year	Western DPS
	all ages > 3 years	Both		15	2	all year	Eastern DPS
	all ages > 3 years	Both		15	2	all year	Western DPS
Eumetopias jubatus	> 5 days to 2	Both	7.p. Ultrasonic imaging to examine	350	1	June-July	Eastern DPS
	months	Both	blubber depth and internal organs	100	4	all year	Eastern DPS
	2 months to 3 years	Both		150	4	all year	Western DPS
	2 months to 3 years	Both		15	2	all year	Eastern DPS
	all ages > 3 years	Both		15	2	all year	Western DPS
	all ages > 3 years						
Phoca vitulina	All ages	Both	8. Incidental disturbance during aerial	500	2	all year	Alaska-wide
Callorhinus ursinus	All ages	Both	surveys, scat collection, instrument	65	2	all year	Alaska-wide
Zalophus californianus	All ages	Both	retrieval, brand resight and capture/sampling activities on Steller sea lions	20	2	all year	Alaska-wide

Table 3. Locations of pup captures (Activity 7) at rookeries in Southeast Alaska for measurements, hot-branding, and sampling (Activities 7a,c,e,h,i) = 2001-2005.

Site	Latitude	Longitude	2001	2002	2003	2004	2005
Forrester Island Complex	54.858N	133.538W	x	x	x	x	
Hazy Islands	55.867N	134.583W	x		x		x
White Sisters Islands	57.633N	136.250W		x		x	x
Graves Rocks	58.25N	136.760W		x			x
Biali rocks	56.71N	135.340W					

APPENDIX 1. JUSTIFICATION FOR AND SUMMARY OF HOT-IRON BRANDING ON STELLER SEA LIONS INCLUDING BRANDING ON OTHER PINNIPEDS

The National Marine Mammal Laboratory (NMML), Alaska Fisheries Science Center, NOAA Fisheries, and the Alaska Department of Fish and Game (ADFG) are presently conducting a collaborative study to determine vital rates, movement patterns, and distribution of the Steller sea lion (*Eumetopias jubatus*) throughout the species' range. The principle tool for obtaining long-term data for these studies is the permanent marking of sea lion pups (and some non pups) with individually identifiable hot-iron brands (letter and number combinations). This effort is a continuation of studies begun in the 1970s and repeated in the 1980s, and 1990s (Table 1). Both groups terminated hot-iron branding (NMML in 1988 and ADFG in 1995) primarily due to budget constraints. However, two workshops hosted by the Steller Sea Lion Recovery Team in the late 1990s and a peer review workshop hosted by the North Pacific Fishery Management Council to review a NMFS ESA Section 7 biological opinion (Bowen et al., 2001) independently recommended that both NMFS and ADFG resume their branding program in order to provide a sufficiently large number of marked animals to calculate age-specific mortality and reproductive rates. It was hoped that these efforts would facilitate determination of reasons for the Steller sea lion decline. Branding programs were re-initiated in 2000 (NMML) and 2001 (ADFG) in response to these recommendations.

In 2001 (ADFG) and 2002 (NMML) both groups began branding all juvenile sea lions captured for movement and physiological studies. This work serves two primary functions. First, by marking all animals handled, sea lions can be targeted for recapture later in life and thereby serve in longitudinal studies of growth rates and physiologic changes in free ranging animals, work that has never before been possible. To date over 85 recaptures of sea lions have occurred for this purpose. Second, although the birth site of a captured juvenile can not always be determined, an age estimate based on tooth eruption can be used so that all marked animals can be included in cohort studies of survival, reproductive rate, and movement patterns. Third, permanent marking of these individuals allow correlations between physiological and morphological parameters and animal condition at capture, and future survival and reproductive probabilities to be explored.

WHY PERMANENT MARKS AND WHY HOT-IRON BRANDS

Hot-iron Brands

Hot-iron branding is the preferred method for producing permanent markings on sea lions safely and effectively. Hot-iron branding has been used extensively as a method to permanently mark pinnipeds, as well as livestock and large birds. The brands are applied with irons (numbers and letters) heated with a portable propane forge. Melin et al. (unpubl.) note their branding of California sea lions (*Zalophus californianus*) is providing a wealth of information on movements and distribution of sea lions, spatial segregation of the sex and ages, and most important a means to estimate and examine the factors that affect survival and natality rates. Branding provides a permanent mark which is not subject to the same problems as plastic or metal tags which become worn and unreadable or fall off. Also, the brand can be easily read from a distance providing much higher resight rates than tags. Also, tag loss is high for Alaskan SSL; of 399 pups branded and flipper-tagged in June 1994, only 2 individuals have been observed with tags after their year of tagging (ADFG unpublished data). Observed tag losses from resights of pups tagged and branded in 2001-2002 (n = 226) suggest *minimum* single tag loss estimates (not accounting for probability of observing whether a tag was lost or retained) of 0.08 and 0.32 by 1 yr of age and 0.19 and 0.40 by 3 yrs of age, for two types of Allflex flipper tags (ADFG unpublished data).

Hot-iron branding has proven effective in practice on Steller sea lions. The ADFG branded 7,046 Steller sea lions with a single character at several rookeries and haulouts in Alaska in 1975-1976 (Table 1). The NMFS, with ADFG and the Soviet Union [Pacific Research Institute of Marine Fisheries and Oceanography (TINRO)], branded 1,489 Steller sea lion pups at Marmot Island, Alaska, and at four rookeries in the Kuril Islands from 1987 to 1989. Russian scientists with KAMCHATRYBVOD, in collaboration with NMFS, branded more than 500 Steller sea lion pups in the Kuril Islands and Commander Islands in 1996. Over 2,700 sea lion pups have been branded by NMML and ADFG since 2000 (Table 2a). At San Miguel Island in California, the NMML and cooperators have branded more than 5,000 California sea lions since 1987 (Table 3). In all of these activities there is no evidence suggesting increased mortality of pups after or caused by branding (Table 2b; Merrick et al. 1996; Melin et al. unpubl.; see below).

Merrick et al. (1996) summarized early studies on Steller sea lions using hot-iron branding. They report that one-month survival of 1,489 pups branded in 1987-89 at rookeries in Alaska and Russia was high (99.8%). From 4-9 months later, no difference was found in mortality rates of branded and unbranded pups from sightings on the beach; 95.8% of the 142 brands observed 5-8 months after branding were legible, and 92.3% of the 26 brands observed 6-7 years after branding were legible.

A mark-recapture study conducted for 3-months post-branding in 2001-2002 at Lowrie Island found weekly survival of branded pups was nearly identical to estimates from a control group of undisturbed/unbranded pups born to 10-11 yr old branded adult females in 2005 (0.987-0.988/wk), and similar to pup survival estimates from other otariid studies. Assuming survival differences between the first 2 weeks post-branding and later weeks was due entirely to the branding event (i.e. no additional natural mortality), potential mortality attributable to the branding event was 0.005-0.006. Although potential effects of maternal age, site and year on pup survival could not be eliminated, the available data indicated significant mortality did not result from the branding event (Hastings et al. *In Review*).

<u>Freeze Brands</u>
Freeze branding has been suggested as a viable alternative to hot-iron branding. However, Merrick et al. (1996) suggest that hot-iron branding is superior to freeze branding because of fewer logistical

problems associated with transporting coolants to remote locations, a better chance of producing permanent, legible marks, and equivocal differences in stress responses of animals (it takes longer to make the freeze mark, but tissues may heal faster).

Freeze branding uses dry ice coolant, Freon 22 (applied to the skin or using a nozzle), or liquid nitrogen (e.g., NMML, 1976). Short contact time of the frozen instrument destroys the pigment-producing hair follicle resulting in un-pigmented white hair during regrowth. Longer contact time destroys both the pigment producing follicle and hair shaft growth follicle, resulting in a hairless or bald brand (Freeman and Lee 1989). Cornell et al. (1979) report on experiments using freeze branding and note that blurring can occur, especially since the iron must be in steady contact with the skin for 45-60 seconds (versus 3 sec. with hot-iron brands) and at 10-15 psi (hot-iron brands are applied with low pressure). Higher pressures resulted in blurred marks. Harkonen (1987) reports on studies using freeze-branding to mark harbor seals. He used brass irons cooled in ethanol and carbon-dioxide ice, applied for 17-22 seconds at a pressure of 4.5 kg. He reported no open wounds and that mortality was no different than non-branded but marked seals. The brands were visible from 700 m for 5 years, but 18% were illegible and 16% were very difficult to discern. Boyle et al. (unpubl.) applied freeze brands to 20 pre-molt New Zealand fur seal (*Arctocephalus forsteri*) pups and monitored the animals at 4, 17, and 41 days. They found that brands were visible for at least the 41 days. But they note that considerable handling time was needed for each application (principally clipping the fur to allow contact of the copper iron with the skin), but that it seemed to cause less stress to the animal than hot-iron branding (but see below re: gas anesthesia).

Troy et al. (1997) used both hot-iron and freeze branding on adult male New Zealand fur seals (*A. forsteri*). In one year, all were freeze-branded, while in the other, all were hot-iron branded. Freeze brands were not legible after the first molt, but became legible following the second molt. Hot-iron brands were legible after the first molt, but 2 had infections 6 months later. They recommended freeze-branding over hot-iron branding because of the lower chance of infection and "over-branding". If first-year resights were necessary, then freeze branding should be supplemented with temporary markings such as tags. Larger males regained territories following the procedure; those that were unsuccessful were significantly lower in weight. The majority of seals under anaesthetic regained territories within 5 days.

<u>Other Marks</u>
Natural marks have also been used to identify animals. This method has been used on a small sample of pinnipeds, but it does not provide information on enough individuals to address questions on vital rates, population dynamics, dispersal, and mortality/natality by age.

Passive induction transponders (PIT tags) have been used on some marine mammals but the disadvantage to this technique is that a "reader" wand must be passed within a few centimeters of the tag in order to identify the animal (read the tag). For most pinniped species this is not a viable option. Additionally, with no external "mark" on the animal it is unknown if the failure to read the tag is due to the lack of a tag, a present but non-functioning tag, or poor technique.

Other marking options that have been used in varying situations with little success are tattooing, web punching, or other anatomical alterations. These techniques have not been considered as viable options for use on Steller sea lions. A more thorough review of other marking techniques can be found in Erickson et al. (1993)

EXPERIMENTAL DESIGN

The hot-iron branding program conducted by NMFS and ADFG will provide age-specific survival rates for the eastern and western DPS's (distinct population segment) of Steller sea lions, with the ultimate goal of identifying the age and sex of highest mortality which may facilitate identification of reasons for decline in abundance. Concomitant to this broader goal will be more detailed determinations of metapopulation age-specific survival rates (e.g., central Gulf of Alaska versus southeast Alaska), age-specific reproductive rates, dispersal from natal rookeries by age and sex, site fidelity, and validation of genetic stock dispersal models. Each topic requires a long-term marking program across a broad geographic range and each requires different study duration and sample size. In general terms, the marking program must occur for multiple years and include hundreds of animals at each marking site each year. More details are provided below.

How Many Years Will Branding Occur?

This is a question that is directly tied to the objectives of the work. At a minimum, in order for basic life history attributes to be obtained, animals should be marked for at least long enough to be able to detect inter-annual variation in survival among different sites. The subsequent resighting effort is crucial here and has to be consistent throughout the life span of the animals. If the inter-annual variation is determined to be low or measurable, branding may only be necessary on intervals, e.g. every 3-5 years. This would maintain a "window" of marked animals within the population that with continued resighting would allow for changes in survival to be documented. However, permanently marking sea lions annually may also be necessary for additional studies examining questions at the rookery level where individual identification is necessary each year.

In general, detecting changes in abundance (i.e., monitoring studies) requires 10 years or more for a species where count or index data are highly variable from year to year and where the rate of change is typically less than 10% per year. The National Research Council (2003) report on the cause of the Steller sea lion decline recommended that adaptive management type experiments would take 5-10 years. These experiments include calculation of vital rates and movement/dispersion between rookeries based on marked animals.

How Many Need to Be Branded

Initial simulation studies suggested that survival estimates with coefficients of variation (CVs) of < 0.125 should result from > 600 animals branded, assuming probabilities of resighting branded 4 and 5 year-old animals were greater than 0.1 and 0.3, respectively. The goal of the ADFG Steller sea lion program is to brand 200 pups per year at up to four rookeries in Southeast Alaska (800 per year total and allowing 1-3 years between branding years), or brand at fewer rookeries per year (600) and alternate rookeries where pups are branded among years. From 2000-2005, 1,475 pups have been

branded at western stock rookeries, while 1,966 at Southeast Alaska (eastern stock) rookeries (Table 2a).
Preliminary analyses of resighting data collected to date on animals branded in 2000-2002 at western stock rookeries (n=627) suggests that the current brand-resight protocol will yield age and sex-specific estimates of survival for pooled rookeries with CVs less than 7%. This level of precision exceeds that predicted because resight probabilities were generally much larger than anticipated, and will enable the detection of differences in survival rates between age, sex or rookery groups that are on the order of 15%. This relatively large detectable difference in survival rate is likely to be reduced as more resight data are collected and as the pool of branded animals increases. If branding continues as planned through at least 2006, it is estimated that CVs of pooled rookery age-specific survival rate estimates will be reduced to approximately 4%.

Likewise the level of branding and resighting effort from 2001-2005 in Southeast Alaska, eastern stock has been sufficient to precisely estimate age-specific survival estimates, and has demonstrated age, sex and natal rookery variation in survival probabilities; and effects of age, sex, and year of observation on resighting probabilities (Hastings et al. 2006). Annual survival probabilities ranged from 0.50-0.70 to age 1, 0.60-0.80 from ages 1 to 2, and up to 0.85-0.95 by age 4-5 yrs of age (Hastings et al. 2006). CVs of survival estimates from all rookeries but Graves Rock averaged 0.032 (minimum = 0.013, maximum 0.054). Only 93 animals were branded at Graves Rock due to the small size of this rookery (98 pups counted in 2002), and given significant variation in survival among rookeries, survival estimates of Graves Rock animals were very imprecise to age 1 (CVs = 0.09 and 0.10 for females and males, respectively) and inestimable after age 1. Resighting rates in this study were high, ranging from 0.65-0.70 for 1-2 yr old females and males of any age, to 0.80-0.90 for 3-5 yr old females (at 3-5 yrs, resighting rate of males was 0.19-0.23 lower than that of females). Reproductive rate studies in 2005-2006 have demonstrated 0.70-0.75 of females $\geq$ 10 yrs of age at Forrester Islands had pups versus 0.41 of 5-6 yr old females and 0.00-0.03 of 4 yr old females (these are proportions seen with pup and not mark-recapture estimates). Age-specific reproductive rates will be estimated with these data using mark-recapture models to account for resighting probabilities of breeders versus non-breeders, and accounting for probability of classifying females correctly as with pup.

Resight probabilities and the standard errors of survival rate estimates may also vary between rookeries in the western stock. Animals branded at Ugamak Island had lower resight probabilities and less precise survival rate estimates than animals branded at other western stock rookeries. This is most likely due to the fact that there is less resight effort within the probable distribution of animals branded at Ugamak than at other western stock rookeries. If the number of animals branded at Ugamak between 2000-2002 were twice the actual, CVs were reduced by only approximately 4% on average. Pooling data across rookeries significantly improved the precision of the survival estimates (CVs less than 0.1) but at the expense of spatial resolution.

Data Collection Procedures and Resight Protocols

ADFG and NMFS have convened two workshops to develop brand resight protocols and methods that can be used by observers from land and water in a consistent manner to assure data compatibility and analysis. To this end, a brand resight computer presentation was developed by ADFG for use by all individuals (within and outside the two agencies) that may be involved in resight efforts. Specific forms with instructions were prepared and distributed for use. Included in the protocol is the recommendation to obtain digital photographs (e.g., use of a Nikon D1 digital camera with 70-300 mm zoom lens) of animals with brands to confirm the observed identification. Numerous resight training excursions have occurred in the field to further develop the protocol and to train naive observers.

Efforts to resight branded animals have been accomplished through the use of dedicated vessel surveys and field camps by both NMML and ADFG, as well as opportunistically by researchers working on sea lion or other research. Dedicated resight efforts consist of observations of sea lions on land by personnel either stationed for several months at field camps on selected rookery and haulout sites or onboard research vessels. Field camps have been routinely occupied during the summer at Forrester and Marmot Islands since the mid 1990s and mid 1980s, respectively, to look for branded sea lions and in particular, collect evidence of their breeding success. Extended field camp observations of sea lions have also been made on Ugamak, Fish, Sugarloaf and Timbered Islands. More recently, video cameras positioned on Chiswell Island and Benjamin Island (SEA), Rogue Reef (OR), and St. George Reef (CA), and soon on Seal Rocks, also provide brand resight effort. Vessel resight cruises are 2-4 weeks in duration and usually occur May – August in the eastern DPS. Sea lions on 50-70 rookery and haulout sites are observed for brands during each of these cruises, which have occurred in British Columbia, Southeast Alaska, Prince William Sound, the Kodiak archipelago, the western Gulf of Alaska (including the Alaska Peninsula and the Shumagin Islands), and the eastern Aleutian Islands as far west as Adugak. Opportunistic brand resighting occurs during all other sea lion field research, including the pup-related research (e.g., branding, counting, physiology, genetics) in July and the foraging/at-sea movement studies using satellite telemetry, in which juvenile and sub-adult sea lions are captured underwater or on-land. Contact with other researchers working in the Aleutian Islands and the Gulf of Alaska, in particular seabird researchers at USFWS and marine mammal scientists at the University of Alaska, is maintained to remind them to look for and record brands if the opportunity presents itself.

Vital Rate Calculation

Melin et al. (unpubl.) estimated survival rates for California sea lions by using the computer program MARK developed at Colorado State University. MARK provides estimates of sighting probability and survival rate for general, open population, capture-recapture models (e.g., Jolly-Seber) and allows models to specify time- and individual-specific covariates for re-sighting and survival probabilities. The model assumes that observed marked animals are representative of those that were alive but not observed. A variety of models can be fitted that allow sighting probability and survival to vary by age, sex, year and interactions of these main effects. For sighting probability, age can be classified based on the sea lion's approximate age at the time of sighting. For survival probability, age is classified based on the age of the sea lion during the applicable survival period. Preliminary calculations by NMFS and ADFG staff using MARK on existing Steller sea lion brand resight data confirm that MARK can be used with these data. Age-specific estimates of natality rate can also be constructed as the proportion of branded females seen with a pup divided by the number of branded females seen during the pupping and breeding season. Also possible is use of MARK, mssurviv or POPAN to estimate age-specific reproductive rates using a robust design and estimation of probability of classifying a female correctly as "with pup" (Schwarz and Stobo 2000, Kendall et al 2002).

Data Ownership and Sharing

NMFS and ADFG staff agreed that all brand application data would be kept in a single data base housed at the NMML, and sharing of resight data between collaborating agencies (NMML, ADFG, Alaska SeaLife Center, and Oregon Department of Fish and Game) would be coordinated. Each agency may keep a separate database consisting of the data in the shared data base plus additional data collected by each agency specific to their needs.

BRANDING METHOD

Branded pups are a sub-set of those pups #1½ months old captured and measured on rookeries during the Steller sea lion breeding season (late May to early July; most branding occurs in late June and early July). Small groups of pups are corralled against cliffs or boulders and taken one-by-one to be weighed, measured, and branded. Branding irons are made of cold-rolled steel (approximately 10mm stock); the dimensions of the largest digits are approximately 5cm wide and 8cm high (Merrick et al. 1996). Each iron is heated red hot in a portable, propane-fired forge and applied perpendicularly to the animal's shoulder with light, even pressure (ca. 5 psi) for 2-4 seconds. Digits are 4-5cm apart to insure clarity of numbers. A 3-digit brand requires about 1-2 minutes to complete. Pups are then released. Pups that are very young(e.g., under 20 kg) are not branded. While this screening might potentially bias survival estimates, very few animals (1-3 per 100 handled) meet the rejection criteria.

The NMML/ADFG hot-iron branding project uses methods originally implemented on southern elephant seals (*Mirounga leonina*) at Macquarie Island in the 1950s (Csordas 1995). That is, each branded Steller sea lion pup has a unique letter and number combination with a different letter designated for each rookery (e.g., T is for Marmot Island; X is for Sugarloaf Island). Thus an animal with T44 was the 44th pup branded on Marmot Island. Presently, all brands are on the animal's left side. NMFS and ADFG decided that one side was sufficient since 1) it reduces handling time and would thus allow a greater overall sample size, 2) typically the brands are large enough to be noticed, even if only on one side, and 3) it seemed likely that as the program progressed, it may be necessary to switch to the right side and start the number/letter sequence over once too many digits were applied to one side (e.g., >five characters).

EFFECTS OF BRANDING ON THE ANIMAL

Pain and Suffering

NMML and ADFG use gas anesthesia (isoflurane) to render the animal unconscious during the branding process. This reduces stress on pups and improves the quality of brands by preventing wiggling during branding. The equipment and techniques used are those developed and described in detail by Heath et al. (1996). This technique has been used extensively with Steller and California sea lions, both adults and pups, and was in fact developed primarily for and during field operations on these species in collaboration with the NMML and the ADFG. Anesthesia is delivered to hand-restrained pups through a mask, which is sufficient for the time requirements of branding. Gas anesthesia has proven safe and effective with sea lions.

Mortality and Growth

Hot-iron branding of California sea lion pups was evaluated beginning in 1987, with a cooperative study between NMML and the SWFSC. From 1987 through 1989, 200 pups were branded annually and the brands were evaluated for healing, brand quality, growth rates of branded and unbranded pups at 3-4 months of age, and resight rate (un-branded pups were tagged). The brands were found to produce good permanent marks and there was no evidence of any mortality of individuals caused by the branding nor was there any significant difference in the growth rates (as measured by weight gain) of branded and control pups (DeLong, unpubl.)

Aurioles et al. (1988) branded 97 California sea lions in 1980-1982. Based on their resight data and mortality calculations, they found that 1) branding did not seem to cause significant mortality, 2) branded pups appeared to be as healthy as non-branded pups, 3) most branded pups (89%, 90%, and 93%, respectively, in each of the 3 years) were alive 6 months after branding, 4) mortality rates for years that pups were branded did not differ from years when no pups were branded, and 5) the number of dead pups present on the rookery in non-branding and branding years indicated that survival was independent of branding.

Beck et al. (1984) analyzed brand and tag data from a study on >1900 gray seals in 1963-1978 and concluded that branding caused no additional mortality compared to just tagging. Numerous other examples on other species are in the literature (Table 3).

A mark-recapture study conducted for 3-months post-branding in 2001-2002 at Lowrie Island found weekly survival of branded pups was nearly identical to estimates from a control group of undisturbed/unbranded pups born to 10-11 yr old branded adult females in 2005 (0.987-0.988/wk), and similar to pup survival estimates from other otariid studies. Assuming survival differences between the first 2 weeks post-branding and later weeks was due entirely to the branding event (i.e. no additional natural mortality), potential mortality attributable to the branding event was 0.005-0.006. Although potential effects of maternal age, site and year on pup survival could not be eliminated, the available data indicated significant mortality did not result from the branding event (Hastings et al. *In Review*).

An estimate of mortality from the branding event can be calculated based on Steller sea lion branding activities from 2000 to 2004 (Table 2b). Assuming that each visit to a rookery for branding is an independent sampling 'event', then a mortality ratio (deaths/branded pups) can be estimated, though in reality the mortality is due solely to the activity (and pup behavior) rather than the branding per se. Mortality rates appear to be highest in southeast Alaska (Table 2b), likely due to handling pups on higher density rookeries laden with cracks and pools. Results suggest that if 800 pups are to be branded each year (400 in each stock), 5 (∀3) pup mortalities could reasonably be expected to occur. Of these, only one would occur in the endangered western stock.

In unpublished studies to assess the effects of branding on Steller sea lion growth, ADFG and NMFS examined 371 juvenile Steller sea lions captured with hoop net or underwater noose techniques during 2000-2003; 27 of these had been branded as pups on natal rookeries. The pups did not differ in mass or length compared to non-branded sea lions of similar age up to 2 years of age (Figures 1 and 2), suggesting there was no effect of branding on subsequent growth. This conclusion was further supported by examination of the distribution of residuals from an analysis of covariance of mass (log-transformed) by sex, branding status (yes/no), and region (natal region for branded pups, region of capture for non-branded pups) with age (log transformed) as a covariate (Figure 3). Though there were significant effects of sex, region and age and the overall model accounted for 71% of variance in mass, there was no significant effect of branding (ANCOVA $F_{(1,370)}=0.008$, $P=0.931$).

<u>Effects of Handling</u>

There are few studies directed at the effect of handling pinnipeds. Baker and Johanos (2002) conducted a study on the effects of research handling on the endangered Hawaiian monk seal (*Monachus schauinslandi*) by analyzing differences in subsequent year survival, migration and condition between handled seals and controls between 1983-1998 (n=549 handled seals). Handling included attaching telemetry devices, blood collecting and tagging. No significant differences in one-year resighting rates, migration rates or condition were noted. They concluded that conservative selection procedures and careful handling techniques have no deleterious effects on monk seals.

Goebel et al. (2003) measured differences in survival to the next year and natality for adult female Antarctic fur seals (*A. gazella*) in which a post-canine tooth was removed under isoflurane gas anesthesia. These females were anaesthetized for >10 min for various procedures, one of which was removal of the first post-canine tooth in a subsample of those processed. No significant differences were found in survival and natality of these adult females when those with and without tooth removal were compared.

Brand Healing and Pathology

New Zealand sea lions (*Phocarctos hookeri*) were hot-iron branded by Wilkinson et al. (2001). They marked 135 adult females and 300 pups (>12 kg); each was also double flipper tagged and implanted with PIT tags. Pups were weighed 12 weeks later and examined for brand healing etc., while adults were examined 1 year later. Of the pups, 10 of 27 (37%) examined had healed brands 12 weeks later, 8 were >95% healed, 3 were 90-95% healed, and 6 were 80-90% healed. 20 of 27 brands were legible. Branding had no effect on pup growth rates after 12 weeks. After 1 year, 10 of 11 pups had completely healed brands and all were legible.

For the adult sea lions, 63 of 98 (64%) examined had healed brands 1 year later, 28 were >95% healed. All brands were legible. Survival rates of the original 135 adult females derived from brands (87%) differed from that assessed from tagging (96%); the authors suggest that tagging overestimates survival rate and that branding is a more appropriate long-term marker for demographic studies than tags.

Duignan et al. (2001) reported on the clinical and pathological assessment of the same animals used in the Wilkinson et al. (2001) study. Approximately 12 weeks after branding they recaptured 23 pups and anaesthetized and weighed them and collected a blood sample. One year later, 16 branded adults were recaptured and anaesthetized. Seven of the pups had completely healed brands, while 16 had incompletely healed brands. Blood was also collected from 19 branded, 24 tagged-only and 11 unmarked pups, as well as all 16 branded and an additional 16 unmarked adults. In four of the pups where healing was "grossly incomplete", there was focal epidermal ulceration with dermal granulation, but all were confined to the brand area in the superficial dermis (<2 mm depth). Serum electrophoretic profiles were indistinguishable between branded, tagged only, or unmarked pups, indicating no acute phase or chronic inflammation even from incompletely healed brands. These results suggest to the authors that any inflammation associated with branding was localized and had no consequence to the sea lion's overall health.

What about "Sloppy" Brands?

Despite the complexity of verification procedures, when research involves charismatic fauna, polemic attitudes can develop that are grounded more in emotive than empirical arguments (Green and Bradshaw, in review). Such a situation occurred when a research project that had been running without incident for 9 years was stopped by the Australian government because of unfavorable public reactions to a hot-iron-branding procedure at Macquarie Island. The issue of the undesirable effects of hot-iron branding surfaced in 2000 when local Tasmania media reported that the hot-iron branding of southern elephant seals (*Mirounga leonina*) was causing physical harm to individual animals. Television and newspapers showed images of unhealed brands and suggested that not only were animals harmed but that the science was flawed because in some animals the brand had not healed properly and that some brands were illegible. (See below for full analysis of this study; van den Hoff et al. in press). The result was a political backlash from the Australian government banning future hot-iron brand activities on animals under their jurisdiction. The government contracted with Dr. N. Gales, a veterinarian, to review the Macquarie Island elephant seal hot-iron branding program. Gales reported (2000) on 646 branded seals examined 1 year after branding, with most (89.5%) 1-3 years old and the remainder 4-7 years old. Only 18.7% of brands were classified as fully healed, while the majority (50.2%) were healed but had some scarring; 19.8% had an unhealed component that was open but with no discharge. 1.7% of the brands had an openly discharging wound indicating infection. Unhealed brands decreased with age.

Brands were applied to both sides of the animal with no reheating of the iron between applications (3 seconds for first, 4 seconds for second). A greater percentage of first brands were completely healed than second brands. Condition of pups when branded affected healing, with more complete healing occurring in pups in better condition. The chronic nature of ulcerations in a large number of animals led Gales (2000) to conclude that branding would adversely affect the welfare and fitness of elephant seals. Almost half were considered readable, but a portion of the brand had to be guessed at. Almost 20% had a component that was completely unreadable.

But the best summary of this Macquarie Island southern elephant study uses the complete data set over a longer time period and is provided by van den Hoff et al. (in press). They monitored 14,000 southern elephant seals branded as pups on Macquarie Island between 1993 and 2000 (Gales 2000 reported on a subset of these animals). Van den Hoff et al. (in press) report that all brand wounds healed by age one and that the number of readable brands increased after the first molt and thereafter; the mean number of brand characters with peripheral skin damage decreased over the same period. They suggested that the seal's hair and skin molting process contributed most to the healing of brand wounds. They also noted that the changes in recorded brand quality were so profound that previously unreadable brands became readable and unhealed brand wounds with peripheral skin damage became healed scar tissue over a short time period.

Even good brands may be mis-read. Melin et al. (unpubl) note that the possibility does exist for mis-reading or incorrectly recording brand numbers. The effect of recording an incorrect brand number will depend on the true status (i.e., alive or dead) of the animal represented by the number that was recorded. Incorrectly recording a number of an animal that is alive should not greatly affect the estimate of survival, but could affect the sighting probability estimate. Schwarz and Stobo (1999) used simulation to show that mis-reading brands creates a positive bias in survival for occasions shortly after branding and a negative bias in survival for later occasions. It is difficult to predict the resulting bias because it would depend on the pattern of errors. Extreme care is taken in recording brand numbers and if there is any uncertainty the brand is not recorded.

Since 2002 both NMML and ADFG have been using digital photographs of all resighted branded animals to increase the accuracy of verification. By comparing photos of the same animal taken at different time periods and using a systematic method of evaluating each digit, the potential for mis-reading the brand is decreased. This method has also proven effective for re-sighting "sloppy" or "smeared" brands. Although the original number may no longer be discernable, the unique mark can still be used for identification and included in survival estimates.

EFFECTS OF BRAND OPERATION ON OTHER SEA LIONS AT THE SITE

The NMML and ADFG are currently collecting data to assess the effect of branding activities on other sea lions at the rookery site, including photographs and counts to assess changes in distribution, age and sex distribution, behavioral parameters (e.g., number of female/pup pairs), and numbers of mortalities. The only other study available to assess the effects of the branding activity is a student project designed to assess the effects of a pup count in the 1980s. Lewis (1987) compared behaviors and distributions of sea lions on Marmot Island in 1984, when there was a pup count (where all adults were moved into the water) and 1985, when there was no pup count. He found that 1) the incidence of pups being trampled during the count was low, 2) that loss and abandonment of pups by females was significantly greater in 1984 than in 1985, 3) a density dependent response in sea lion displacement from disturbed rookeries: the greatest displacement of non-pups occurred on the largest rookery, which was disturbed twice in 3 days, while less displacement occurred on the smaller rookeries which were disturbed only once, and 4) that females became increasingly aggressive and territorial for about 1 week following the counts, then this subsided. Pup counts caused an increase in the frequency of sea lions stampeding from the rookery in response to natural events for about 3 days, then subsided. He suggested that pup counts be conducted during times and tidal conditions when the number of non-pups present is lowest, that refuge areas with no disturbance be established within rookeries, that counts not be conducted when rookery area is small because of storms and pooling of water (where pups may drown), and that counts be conducted between 20 June and 1 July (after most pups are born and prior to them entering the water).

It should be noted that the behavior of humans during a pup count and pup branding are not the same – pup counts involve walking through the entire rookery (at least during Lewis' study) and effectively disturbing and 'moving' every animal. During branding the entire rookery is not disturbed; if the branding activity is restricted to a few areas on the rookery, then pups out of sight are not disturbed.

CONCLUSIONS

Determination of age-specific survival and vital rates is essential to evaluating multiple hypotheses proposed for the Steller sea lion population decline. Of all marking methods available, only branding produces a permanent mark that can be easily and reliably read from a distance. This allows for higher resight rates with less disturbance than obtainable with other marking methods.

Though freeze-branding has been used in some pinniped studies, difficulties with logistics, brand application and legibility exist and thus hot-branding remains a preferable method for Steller sea lion studies. Mortality related to branding at rookeries is low, and a result of pup behavior and anesthesia complications rather than branding. There seem to be no longer term consequences of hot-branding on growth rates or survival, and brand-associated wounds generally heal within the first year of life. Current studies of hot-branded Steller sea lions in the U.S. will provide survival rate estimates with high precision and minimal impact on individuals or populations.

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Table 1. Year, location, and number of Steller sea lion pups hot-iron branded in Russia and the United States.

Year	Location	Number branded	Year	Location	Number branded	Year	Location	Number branded
1975	Marmot	598	1998	Antsiferov	100	2002	Forrester	141
	Sugarloaf	719		Brat Chirpoyev	100		Graves Rock	50
1976	Cape St. Elias	23		Iony	150		Iony	100
	Fish (Wooded)	29		Kozlov	50		Kozlov	50
	Marmot	3669		Medney	87		Marmot	90
	Outer	249		Raykoke	100		Medney	85
	Seal Rocks	316		Srednego	100		St. George Reef	141
	Sugarloaf	1443	1999	Antsiferov	50		Sugarloaf	105
1986	Rogue Reef	56		Brat Chirpoyev	100		White Sisters	126
1987	Marmot	351		Kozlov	50		Yamski	150
	Rogue Reef	55		Lovushki	100	2003	Antsiferov	100
1988	Marmot	400		Medney	100		Brat Chirpoyev	100
	Rogue Reef	92		Raykoke	100		Forrester	291
1989	Brat Chirpoyev	200		Srednego	80		Hazy	101
	Lovushki	200	2000	Marmot	107		Lovushki	77
	Srednego	200		Sugarloaf	151		Seal Rocks	100
	Raykoke	139		Yamsky	90		Raykoke	100
1994	Forrester	399	2001	Antsiferov	100		Rogue Reef	190
1995	Forrester	400		Brat Chirpoyev	76		Srednego	100
1996	Antsiferov	100		Fish	32		Ugamak	150
	Brat Chirpoyev	100		Forrester	286		Medney	54
	Kozlov	50		Glacier	14	2004	Marmot	75
	Medney	100		Hazy	217		Sugarloaf	110
	Lovushki	100		Iony	143			
	Raykoke	100		Lovushki	100		**TOTAL**	**16415**
1997	Antsiferov	100		Medney	95			
	Brat Chirpoyev	100		Raykoke	100			
	Kozlov	50		Rogue Reef	180			
	Lovushki	100		Seal Rocks	75			
	Raykoke	100		Ugamak	177			
				Yamsky	81			

Table 2a. Summary of brands applied to Steller sea lion pups in the United States.

Western stock				Eastern stock				
Year	Location	Number branded	Lead letter	Year	Location	Number branded	Lead letter	Total
1987/88	Marmot	750		1994/95	Forrester	800?	F	
2000	Marmot	107	T	2001	Lowry	286	F	
	Sugarloaf	151	X		Hazy	213	H	
2001	Ugamak	175	A		Rogue Reef	180	R-following	
	Fish	32	E	2002	Lowry	141	F	
	Seal Rocks	75	J		Graves Rock	50	V	
2002	Marmot	89	T		White Sisters	126	W	
	Sugarloaf	105	X		St. George Reef	141	Y-following	
2003	Ugamak	150	A	2003	Lowry	291	F	
	Fish	0			Hazy	101	H	
	Seal Rocks	100	J		Rogue Reef	190	R-following; open circled	
2004	Marmot	75	T	2004				
	Sugarloaf	110	X					
	Total (all years)	1919			Total (all years)	2519		4438
	Total (2000-04)	1169			Total (2000-04)	1719		2888

Table 2b. Estimates of mortality rates (*m*, mortalities per animal handled) for Steller sea lion as a consequence of pup branding activities shown in Table 2a. All mortalities resulted from suffocation or drowning from pups piling when corralled, none resulted from branding. Ratio and standard error determined using ratio estimator method. "Events" are the number of rookeries visited for branding.

Group	Stock	Period	Mortalities	Handled	*m*	*SE (m)*	Events
NMML-AEP[1]	western	2000-2004	2	1169	0.002	0.002	13
NMML/ODFW[2]	eastern	2001-2003	2	511	0.004	0.002	3
ADFG[3]	eastern	2001-2003	14	1208	0.010	0.005	7
Pooled			*18*	*2703*	*0.007*	*0.002*	*23*

[1]National Marine Mammal Laboratory-Alaska Ecosystems Program
[2]National Marine Mammal Laboratory-California Current Program/Oregon Department of Fish and Wildlife
[3]Alaska Department of Fish and Game

Table 3. Summary of studies in which branding was used on pinnipeds.

Species	Year	# pups	# other	Where	hot/freeze	Who
Steller sea lion	1977-present	>16,000	>300	throughout range	Hot	NMML, ADFG, Ru
CA sea lion	1987-present	>5,000		San Miguel	Hot	Melin et al.
CA sea lion	1980-1982	97	0	Baja CA	Hot	Aurioles et al.
New Zealand sea lion	2000	300	135	New Zealand	Hot	Wilkinson et al.; Du
Northern fur seal	1912; 1960s-70s	>50,000	variable	Pribilof Islands	hot and freeze	FSI; Erickson et al.
New Zealand fur seal	1994	20	0	Otago Peninsula, New Zealand	Freeze	Boyle et al.
New Zealand fur seal	1992-1993	0	19 adult males	Kangaroo Island, South Australia	1992-freeze 1993-hot	Troy et al.
Cape fur seal	1947-1948	600	600	??	Hot	Carrick and Ingram
Southern elephant seal	1993-2000	>14,000	0	Macquarie Is., Tasmania	Hot	van den Hoff et al.;
Southern elephant seal	1951-1961	4,910	?	Macquarie	Hot	Csordas
Southern elephant seal	1949-1953	1,486	0	Heard Is.	Hot	Csordas
Southern elephant seal	1929	?	?	South Georgia	Hot	Mathews 1929 (in C
Southern elephant seal	1945-1947	several	0	Campbell is.	Hot	Sorenson (in Csord
Southern elephant seal	1949-1953	>1,400	48	Heard Is.	Hot	ANARE (in Csorda
Weddell seal	1932	243	243	??	Hot	Lindsay (in Csorda
Gray seals	1963-1989	4,448	0	Sable Is.	Hot	Beck et al.; Schwar
Ringed seals	1971	0	121	Herschel Is., Yukon	hot (electric and forge)	Smith et al.

Appendix 2. Resources needed to Accomplish Objectives – NOAA Cooperative Agreement #NA04NMF4390170

APPLICATION FOR FEDERAL ASSISTANCE

STATEMENT OF WORK

STELLER SEA LION RECOVERY INVESTIGATIONS

1 JUNE 2004 TO 31 MAY 2008

ALASKA DEPARTMENT OF FISH AND GAME

DIVISION OF WILDLIFE CONSERVATION

333 RASPBERRY ROAD

ANCHORAGE, AK 99518

(Attention: Lorrie Rea)

INTRODUCTION

Steller sea lions (*Eumetopias jubatus*; SSLs) are the largest member of the family Otariidae. Although SSLs range from California north and west to Japan and Russia, Alaska is the center of distribution and contains the majority of the world population. These animals inhabit waters over the continental shelf and beyond and haul out on land at specific traditional sites. The National Marine Fisheries Service (NMFS) recognizes an eastern and western stock of Steller sea lions in the United States, based primarily on genetic data (Bickham *et al*. 1996, Loughlin 1997), with a division lying at Cape Suckling, Alaska (144^{o} west longitude). SSLs were previously abundant along the North Pacific Rim, however, over the past 25+ years the Alaskan segment of the western stock declined by over 80%. The western stock is now classified as endangered under the Endangered Species Act and as depleted under the Marine Mammal Protection Act and the eastern stock is listed as threatened. A population viability analysis (York *et al.* 1996) suggested the U.S. portion of the western stock could become extinct in less than 100 years if 1985-1994 population trends persisted. In contrast, the Alaskan portion of the eastern stock, located in Southeast Alaska, has nearly doubled over the past 20 years (Calkins *et al*. 1999).

In November of 2000, the NMFS issued an Endangered Species Act – Section 7 Consultation Biological Opinion and Incidental Take Statement concluding that proposed Bering Sea/Aleutian Islands and Gulf of Alaska groundfish fisheries actions and their cumulative effects would be likely to jeopardize the continued existence of the western population of Steller sea lions. The Reasonable and Prudent Alternative provided by the 2000 Biological Opinion met with considerable criticism and more recent data analysis revealed new information not previously available. Therefore, in October of 2001, NMFS again issued a Biological Opinion relying on the most recent data, scientific reviews and comments.

The 2001 Biological Opinion stated that there is still no clear leading hypothesis explaining the continued decline of the western population of Steller sea lions (NMFS Biological Opinion, p. 178). Additionally, the Opinion stated that qualitative information continues to be lacking on the habitat requirements of Steller sea lions and therefore decisions have primarily been based on the judgement of scientists. It is apparent that additional data is required for a clearer understanding of Steller sea lion natural history, vital rates, diet and other factors possibly relating to the continuing decline and the lack of recovery of the western population of Steller sea lions.

In December 2002, the ninth circuit court ruled the agency's 2001 Bering Sea Groundfish Biological Opinion (BiOp) invalid due to inadequacies in its use of telemetry data and its analsyis of fishing effects in protection zones. Also in December 2002, a panel of experts convened by the National Research Council of the National Academy of Sciences concluded that no existing hypothesis could be fully excluded as a possible cause of the current decline of the western stock. The panel felt that top-down sources of mortality such as predation, incidental take, subsistence, illegal shootings and pollution or disease pose the greatest threat to the current population. Furthermore, they recognized that continued emphasis should be put into demographic studies indicative of the status of the population.

Several areas of research have been identified as particularly important to understanding Steller sea lion biology and the effect of fisheries on individual and population health and condition. These include (but are not limited to) determination of current vital population statistics of both the western and eastern populations of sea lions, collection of data on the location and diving behavior of juvenile sea lions during different times of the year to determine habitat use and potential areas of overlap with fisheries, analysis of diet and the nutritional condition of Steller sea lions to assess the hypothesis of food limitation as a cause of continued population declines and an assessment of other sources of possible sea lion mortality (disease, pollution, predation, incidental catch etc.).

The majority of the data available for review in the Biological Opinion was collected from lactating adult female Steller sea lions and pups during early lactation (e.g pups up to approximately 5 weeks of age). Although reduced food availability resulting in low juvenile survival and periodic low adult survival plus reduced fecundity is the favored hypothesis to explain dramatic declines in the *western* sea lion population during the 1980's (Calkins and Goodwin 1988, York 1994, Merrick 1995, NMFS 1995, Calkins *et al*. 1998, Pitcher *et al*. 1998), most of the research conducted in the 1990's does not support the hypothesis that food was limiting for early lactation breeding females and their pups in the western stock, at least during the period that the research was conducted (1992-1997). Additionally, no large mortality events have been documented that could explain the disappearance of the juvenile age classes.

Research comparing the declining western population and the increasing eastern population indicates in virtually every parameter tested, that there was no indication of lower nutritional status in the declining western stock (Adams *et al*. 1996, Brandon *et al*. 1996, Davis *et al*. 1996, Rea *et al*. 1998, Swain and Calkins unpublished data, Andrews unpublished data). SSLs from the declining western stock did not demonstrate behaviors expected from nutritionally stressed animals (Milette 1999). These findings are not conclusive evidence that reduced food availability was not a factor in the original decline or that undernutrition is not currently limiting the western stock. It is possible, as the population is at a low level, that factors other than nutrition have driven more recent declines or have prevented substantial recovery. This fact was emphasized in the National Academy report. The other mortality sources could include predation, incidental take in fisheries, subsistence harvests, contaminants and disease, and illegal shooting. Another possible explanation for the lack of findings supporting nutritional stress in the western stock as a cause of the decline was that sampling (both physiological and behavioral) was done on rookeries populated by individuals that were well nourished. The nutritionally stressed segment of the population may have been either at sea or on haulouts and not been sampled. Also, nutritional stress could be occurring during other seasons or affecting other age classes than those sampled.

Until recently, life history information about Steller sea lions has come from studies conducted on adult breeding females and their pups due to the accessibility of these animals to capture using remote immobilization. However, this may not be a season or life history cohort at which animals are influenced by competition from commercial fishing efforts, and it has been suggested that the juvenile age classes may be the most vulnerable. Therefore, research by the ADFG Steller sea lion program over the last 6 years has concentrated on juveniles. This required the development of a safe and efficient method of capturing juveniles with as little disturbance as possible. This was especially critical because of the desire to handle animals without disturbing other sea lions on the haulout.

Since the introduction of new underwater capture techniques in 1998, over 400 juvenile sea lions, up to 2 to 3 years of age, have been safely and efficiently captured. Captured sea lions are held for short periods of time to measure body condition, collect blood and other tissue samples, and to deploy remote monitoring devices such as satellite-linked time depth recorders (SDR's) that provide information on diving behavior and geographical location of movements. This capture method has permitted us to collect extensive data on a large sample of individuals. This information is being integrated into a long term research program to address basic questions regarding diving ability, the use of specific geographical areas by juveniles, forage items that may be of particular importance in the diet of these young animals, animal condition, and at what time of year these animals become nutritionally independent from their mothers, and thus may be most susceptible to low food availability.

PROPOSAL SUMMARY

The major emphasis of our proposed study plan builds on long-term work initiated and perpetuated during the last few years; to evaluate the hypothesis that the endangered western stock of Steller sea lions (SSLs) is continuing to decline because of nutritional stress that heavily impacts juveniles up to 3 years of age. This stress may be unique to the western stock and thus comparisons to the eastern (non-declining stock) are useful for recognizing possible cause. Fundamental to those questions is the examination of the basic biology of Steller sea lions. Within that context a diversity of studies have been employed which may illuminate other possible causes of the decline. The ADFG Steller sea lion program has been capturing juvenile animals during their first 2 to 3 years of life, obtaining data on growth, body condition, weaning status, and health and disease, and attaching satellite-linked time depth recorders (SDRs) to obtain data on dive behavior and movements. Two to three research cruises to capture juvenile SSLs, mark newborn pups, and resight marked animals of all ages will be scheduled during different seasons over the duration of the award period (May, June, September, and February for example). This field-work allows us to assess seasonal and age related differences in survival and reproduction, to investigate sea lion nutrition and body condition through an exhaustive suite of sample collections, and to work collaboratively with other agencies and universities on large integrated projects. Comparisons of these parameters are continuing to be made between the thriving population in Southeast Alaska and the depressed and declining population in western Alaska in order to evaluate the hypothesis that undernutrition is limiting the recruitment of juvenile SSLs into the western Alaska population. Capture trips will occur in Southeast Alaska, Prince William Sound and the Aleutian Islands.

An integral part of this work is the continuation of long-term research focusing on the marking of individuals for the collection of population demographics. This involves annual pup branding and brand-resighting trips in southeast Alaska. Simultaneously, the National Marine Mammal lab is conducting similar work in western Alaska. Approximately 300 - 500 pups were branded each year in southeast Alaska during the summers of 2001, 2002 and 2003, and another group will be branded during each year of this contract. This work is basic to virtually all of the current research that requires data collected on individual life histories and is strongly advised by research review panels, the Steller Sea Lion Recovery Team, and the Marine Mammal Commission. The additional marked animals in the population will substantially increase the number of resightings each year and therefore we are including adding additional effort to the resight work under this contract. As in the past, we anticipate that this element of our program will increase in logistical requirements over time.

We are continuing to coordinate our research efforts whenever, and wherever possible with focused fisheries research, sea lion abundance surveys, habitat use projects, and contaminant studies undertaken by other research groups (e.g. NMML, NMFS/Auke Bay Laboratories, UAF Southeast and the UAF Gulf Apex Predator project) to provide assessment of diet, body condition and habitat use

BACKGROUND/ NEED FOR FEDERAL FUNDING

As noted in the Introduction, the Steller sea lion was listed as a federally endangered species in areas west of Cape Suckling in 1997. The Eastern stock population is listed as threatened. These actions, particularly in regard to the western stock continue to focus a great deal of attention on the status of sea lions in Alaska. Under Section 7 of the Endangered Species Act, federal agencies are required to ensure that federal actions such as the setting of fishery limits and regulations are not likely to jeopardize the continued existence of an endangered species. The hypothesis that the decline of SSLs could be related to adverse interactions with the Alaskan ground fish fisheries caused the NMFS to establish closures on areas and fisheries in Alaska that could ultimately have a negative effect on the livelihood of Alaska fishermen (Ferrero and Fritz 2002). This issue was used as an argument during Senate deliberations in summer 2000 to increase the amount of federal funding available for Steller sea lion research in fiscal year 2001.

The mystery of whether the Alaska ground fish fishery is influential in the past and current decline of the SSLs remains unsolved. Therefore, in the arena of uncertainty, NMFS management is compelled to err on the side of conservatism and continue to enforce closures on Alaska fisheries. An. essential part of the Steller sea lion program at ADFG is the collection of data necessary for the managers to make defensible decisions. Federal funding is requested to continue our work involved in the long term projects of marking and following animals throughout their lives, collecting intensive physiological measurements, examining animal behavior, and producing methods and techniques for use by other researchers investigating similar questions throughout the sea lion's habitat.

By merging this information we hope to increase our understanding of the biology of SSLs and the threats to the population.

PROJECT GOALS AND OBJECTIVES

Our research goals will be accomplished through the successful completion of the following primary research objectives and their specific tasks. Most of this work is a continuation of long-term projects initiated during previous grant years. The essence of good science is founded on the ability to learn from previous work and adjust accordingly. Therefore the objectives listed in this four-year document will almost certainly be modified as new data and new techniques continue to shape our work. Nevertheless some of the work listed here will occur on a timeline that is greater than the period of the requested grant. All of the projects listed below fall under the general objective or our program, to investigate research questions that collectively will improve our knowledge of the biology and habits of Steller sea lions and thereby contribute to their conservation. The objectives are referred to under the Statement of Work for each year and new topics are noted as others are completed. The personnel listed at the end of the proposal are all involved in carrying out the tasks and their specific duties are included. Major products when distinct to the task are also included.

1. **Describe the ontogeny of diving behavior and nutritional independence in juvenile Steller sea lions**. ADFG has developed an efficient method of capturing juvenile SSLs up to 3 years of age. Data collected on animals captured since 1998 and instrumented with satellite-linked transmitters is being used to address numerous questions about sea lion behavior, independence, and movement patterns. Capturing the animals allows us to obtain blood, blubber and whisker samples to determine if individuals are obtaining energy from suckling and/or from prey and to collect teeth for aging the animals. These data will provide information on the ability and limitations of juvenile SSLs to independently obtain prey at various times of the year (March, May, September and November for example). The location and dive profile data collected will provide managers with information on juvenile habits that may be useful in managing fisheries in a way to enhance juvenile survival and possibly avoid conflicts. A large collection of virology, parasitology, and bacteriology swabs are also collected for different studies of disease. The tasks described involve merging observations of animal behavior with physiological indicators of animal condition.

Task 1A. **Final analysis of diving behavior data from SDR deployments to document the development of diving patterns of juvenile sea lions in Prince William Sound (n=14) and southeast Alaska (n= 58) between March 1998 and December 2000**. A manuscript entitled "The Ontogeny of Diving in Steller Sea Lions" has been accepted with revisions to the journal Marine Mammal Science. This paper should be published during first year of this proposal.

Task 1B. **Developing indices of weaning and identification of diet composition.** When juvenile sea lions begin ingesting significant amounts of prey species, the fatty acid composition of blubber and blood samples will change to reflect the incorporation of individual fatty acids that are absent or under-represented in milk lipids. Laboratory analysis has been completed on over 450 juvenile Steller sea lions to determine the fatty acid composition of blubber and recently ingested milk. During 2004, samples from an additional 50 animals will be analyzed to complete this work. Preliminary data have been presented at three workshops or conferences and two manuscripts will be submitted for peer review during the 2004/2005 funding period. The first manuscript, "Blubber fatty acids reveal regional and age class differences in the diet of young Steller sea lions in Alaska" will describe the qualitative differences seen among fatty acid profiles of juveniles Steller sea lions captured in Southeast Alaska, Prince William Sound and the Aleutian Islands. The second paper, " Seasonal, sex and age-class differences in the diet composition of juvenile Steller sea lions in Prince William Sound" will use quantitative fatty acid signature analysis (QFASA) to model the diet of juvenile Steller sea lions captured in this region of Alaska. Quantitative fatty acid signature analysis, developed by Dr. Sara Iverson and colleagues at Dalhousie University, allows the diet of a predator to be inferred based on its fatty acid composition and the distinct fatty acid composition patterns in the lipids of prey species. Blubber samples, along with samples of ingested milk collected from the sea lions' stomachs, will be used to compare the fatty acid signature (FAS) of individual sea lions based on age, season and geographical area with a large library of FAS from prey species collected during previous studies in Prince William Sound. To date, this project has been jointly funded through a North Pacific Marine Research Institute grant to Lorrie Rea and John Kennish (July 2002 to May

2004) and ADFG. During the final data analysis and publication stages of this project, we will provide full salary support for a Post-doctoral Research Associate (Dr. Carrie Beck) to work with Dr. John Kennish (lipid chemist) at the University of Alaska Anchorage. Dr. Beck brings specialized skills to the project including the collection and processing of fatty acid samples using gas chromatography, and the analysis and interpretation of fatty acid signatures in relation to a predator's role in the marine food web. As data becomes available through other researchers undertaking fatty acid analyses for prey in other regions of Alaska, we will continue to make efforts to collaborate in joint analysis of data sets in attempts to determine diet composition of Steller sea lions captured in other regions of Alaska. We also plan to continue our investigations on the diet composition of lactating females at different times of the year. As our collection of ingested milk samples from captured juveniles increases, we will gain a more complete representation of the FAS of the milk diet, with which we can model the prey composition of the diet of lactating females using a similar QFASA approach.

We propose to further develop this use of fatty acid signatures as an index of nutritional independence during the upcoming funding periods by investigating the relationship among fatty acid signatures of ingested milk, serum and blubber. Lipids circulating in serum will best represent the most recent meal ingested and thus provide a much more discrete signature analysis of recent diet than does the more integrated blubber signature.

Task 1C. **Stable Isotope analysis.** Laboratory analysis has underway to determine changes in stable isotope patterns laid down over time in sea lion whiskers. Preliminary analyses of stable isotope signatures along the length of whiskers collected from juvenile sea lions (ages 3 to 24 months) suggest that relative changes in the ratios of carbon and nitrogen isotopes may prove to be a valuable index of shifts in diet such as that expected during the weaning period. This work has been undertaken in collaboration with Dr. Sean Farley (ADFG) and researchers from the Crustal Imaging and Characterization Laboratory, USGS in Denver CO. To date over 1700 samples have been analyzed which represent timelines of isotopic change in the whiskers of over 50 Steller sea lions. Preliminary results from this study have been presented at 3 conferences. During the upcoming year, we propose to undertake an intensive C/N isotope analysis schedule focusing on samples remaining from previous captures and samples collected during this year's field captures. We plan to report the final results of the first stage of this work in a collaborative, peer-reviewed publication during the 2004/2005 period, which will be titled "Stable Isotope Signatures Indicate Weaning in Steller Sea Lions". Due to the early successes using this technique this project will be continued throughout the period of this agreement to help to identify the weaning status of each sea lion that is captured and if weaned, to estimate when that weaning occurred.

2. **Evaluate Steller sea lion movement patterns to assess habitat use and dispersal.** Through the use of satellite-linked radio transmitters attached to the dorsal pelage of SSLs, we are continuing to record the movements and behavior of sea lions. We are using this information in the context of other environmental factors to examine hypotheses on sea lion movement relative to prey distributions and population stock.

Task 2A. **Analysis of location data from SDR deployments to document movement patterns of juvenile sea lions in Prince William Sound (n=14) and southeast Alaska (n= 58) between March 1998 and December 2000**. Summary analyses of this data set were presented at the Biennial Marine Mammal Meeting in Dec. 2003 and will be submitted for publication in a peer-reviewed journal during the 2004-2005 grant period.

Task 2B. **Collaborate with other funded researchers to deploy additional SDR's during juvenile capture trips**. We are in the final stages of a collaborative project with researchers at The University of Alaska, Anchorage to deploy satellite instruments designed by the Sea Mammal Research Unit on juvenile SSLs in both stocks. This work was part of a graduate thesis by Michael Rehberg, an employee of the Steller sea lion program and his advisor at UAA, Jennifer Moss Burns. Michael will be defending his thesis during the first year of this proposal. Additionally, we are collaborating with Drs. Ben Wilson and Mary-Anne Lea from the University of British Columbia to deploy their instruments in Southeast Alaska. They are working with funding received from the Marine Mammal Research Consortium and by working cooperatively with our group are able to capture sea lions for instrument attachment. The deployment of their instruments will be complete during the FY 2004 proposal year, however we will continue to solicit other collaborations involving satellite telemetry, thereby creating a more efficient use of federal dollars distributed to researchers. During a previous funding year we collaborated with NMFS researchers at the Auke Bay lab to merge sea lion behavior with prey (fish) distribution. The instrument deployment for this work is finished and the first year of this grant will support the data analysis and initial writing of this report. A joint publication describing juvenile sea lion behavior in the context of prey distribution will be completed during the second year of this proposal.

3. **Evaluation of nutritional limitation in juvenile Steller sea lions in the western Alaska population**
Underwater capture techniques have allowed us to obtain body measurements and tissue samples to evaluate growth, body condition, health, and weaning status of juvenile SSLs from the western Alaska population in relation to those from Southeast Alaska where the population has been increasing. We have captured and processed over 400 juvenile sea lions in Southeast Alaska and Prince William Sound and therefore have made progress in developing a comparative database. These studies include analysis of body fat content using the deuterium dilution technique, collection of body morphometrics and blubber depth as an index of body condition, and blood chemistry and hematology, in addition to investigating the use of stable isotope signatures in the whiskers and fatty acid signature analysis of blubber lipids to identify changes in diet source (i.e. weaning markers). These collections also allow for the continued development of a serum archive for retrospective disease screening to address the interactions between nutrition and disease state in these animals. The majority of samples have been collected in PWS and southeast Alaska over recent years. In 2004-2007 our efforts will concentrate on two objectives: 1. Gathering comparable samples from juveniles in the western population, including the Aleutian Islands, and 2. Investigating morphometric changes in known-age individuals recaptured in Southeast Alaska and Prince William Sound.

We propose two to three research capture excursions /year during this contract period which will be distributed between areas and seasons and will be coordinated with other Steller sea lion researchers to reduce disturbance and unnecessary duplication. If our attempts at recapturing marked sea lions proves successful, various new avenues of investigation will be possible in the future that require the repeated study of known individuals or the retrieval of archival instruments. This is ongoing work and will be incorporated into a project evaluating Nutritional Limitation in Juvenile Steller Sea Lions. This work is anticipated to continue throughout the timer period covered by this proposal.

Task 3A. **Analysis of body composition (percent body fat) data collected from juvenile sea lions using the deuterium dilution technique**. Analyses of the percent body fat of over 400 juvenile Steller sea lions (ranging from 2 to 26 months of age) have been completed to date and preliminary analyses of this data have been presented at 4 workshops or conferences to illustrate how body fat content differed between geographic regions and with age of the animal. We will continue to collect data on body composition of juvenile sea lions during the capture excursions proposed for 2004-2007, with particular emphasis on increasing sample size of animals captured in the Aleutian Islands. These data will enable us to identify specific times during the year when animals exhibit poorest body condition, and to assess whether animals in the western stock are in poor body condition compared to those animals captured in the area of increasing population. These data will also be used as a factor in the development of a physiological index of weaning (along with fatty acid signature and stable isotope analyses) since it is expected that percent body fat content will decrease when animals are forced to switch from a higher fat content milk diet to a lower fat content diet of fish prey at weaning.

Task 3B. **Assessment of seasonal and age related changes in morphometrics and nutritional state of juvenile sea lions in the eastern and western populations. Hookworm studies**. We propose to continue collecting data on body mass and other morphometrics (standard length and girth measurements), blood chemistry and lipid concentrations during all capture trips during this funding period. Preliminary analysis of blood metabolite levels in juvenile Steller sea lions was presented at an international conference in December 2001. These data suggested that a significant proportion of animals sampled at 9, 11 and 23 months of age exhibited evidence of fasting, based upon metabolite levels established in captive fasting experiments on this species. With additional samples collected during the upcoming juvenile capture excursions we expect to balance our sample sizes adequately to establish whether there are differences in the proportion of fasting animals in the eastern versus western stocks and to identify specific times of the year when animals are exhibiting this fasting metabolism. We plan to augment these blood chemistry analyses with complete blood lipid profiles (including triglyceride, phospholipid, and cholesterol components) measured using thin layer chromatography and flame ionization detector techniques (Iatrascan MK6 instrumentation).

During the 1st year of this proposal we will be attempting to re-capture sea lions handled during previous field trips. These animals will allow us to examine changes in morphology over time; indicators of condition in rapidly growing animals. In addition we will be examining the incidence of hookworm in the population and its effect on new born sea lions. Hookworm was recently shown to significantly increase the mortality of young California sea lions. This project, if successful will continue during the remaining years of this proposal.

Task 3C. **Analysis of biochemical, histochemical and ultrastructural properties of muscle tissue to assess nutritional condition of juvenile Steller sea lions.** The process of prolonged nutritional stress or starvation strongly impacts skeletal muscle, which represents the body's main store of protein and roughly one-half of the lean body mass of terrestrial mammals. Previous studies have shown that the effect of starvation on a muscle's mass, fiber size, and histochemical and biochemical properties are dependent upon its pattern of contractile activity and oxidative capacity (Stegall 2001). Thus, in this study, a continually active swimming muscle from the pectoralis complex, and a less continually active swimming muscle from the hindlimb complex of captured and stranded juvenile Steller sea lions will be compared. This research was delayed pending the availability adequate laboratory facilities, however, muscle samples from over 50 sea lions have been archived and laboratory analysis is scheduled to begin in September 2004. The goals of this study are to characterize the body condition of juvenile Steller sea lions by (1) using histochemical and transmission electron microscopy techniques to characterize muscle fiber type compositions and fiber atrophy for each muscle, and (2) using biochemical techniques to characterize each muscle's metabolic status. These techniques, which are based upon studies of starvation in wild and domestic animals, have been applied to assess the nutritional status of harbor porpoises (Stegall 2001), and thus we are encouraged that they would also be sensitive to muscle degradation in sea lions if it were present. This research will be undertaken with collaborators at the University of Alaska, Fairbanks (Dr. Michael Castellini) and the University of Alaska, Anchorage (Dr. David Pfeiffer) and Ms. Stegall will lead her co-authors in the peer-reviewed scientific publication titled "The effects of nutritional stress on skeletal muscle of juvenile Steller sea lions, *Eumetopias jubatus*." This project will also continue previous research directions on muscle biochemistry development undertaken during the past 3 years by UAA collaborators (Richmond and Burns) since interpretations of the impacts of nutrient limitation must be made in the context of know changes to muscle chemistry and morphology with age.

Task 3D. Elemental signature analysis and evaluating the nutritional impact of micronutrient limitation. During 2003 we initiated a pilot project using laser ablation ICP-mass spectrometry to measure the elemental composition of sea lion whiskers. This project is in collaboration with Dr. Sean Farley (ADFG) and Dr. Ian Ridley (USGS, Denver). Through our preliminary studies we have determined that it is possible to monitor changes in over 30 elements in very small increments along the length of the whisker to determine changes in deposition of elements in this tissue over time. Many of these elements are known to be important micronutrients in mammalian nutrition and thus are of interest to monitor potential limitations in the availability of specific elements or compounds in the sea lion diet during development. Since laser ablation does not impact the use of these same whiskers for C/N stable isotope analysis, we are able to interpret these changes in micronutrient deposition, in context with changes in diet as indicated by the carbon and nitrogen isotope signatures described above. This work will be expanded to include other tissues (such as muscle), and will be coordinated with research on muscle biochemistry and morphology to determine the possible link between poor muscle development (if any) and micronutrient content of these tissues. This project also holds potential for development of a retrospective study using tissues archived from collections in the 1970's and 1980's.

Task 3E. Identification of nutritional biomarkers. During the next 3-year funding period we propose to develop research projects that identify and quantify a set of nutritional biomarkers to specifically aid in identifying whether sea lions are feeding on solid prey or nursing. Two such biomarkers that will be investigated are trimethyl amine oxides (TMAO) and gastric enzymes. TMAO is a compound found in high concentrations in many fish and has been found to be a reliable indicator of fish ingestion in Weddell seals and gastric lipase concentrations have been show to alter significantly around the time of weaning in other mammals. Blood and gastric juice samples are currently available in our sample archived for these analyses.

Task 3F. **Development of thyroid hormones and leptin as indicators of metabolic condition**. ADFG will continue to collaborate with scientists from the Alaska SeaLife Center in the ongoing analysis of thyroid and leptin hormones from approximately 250 blood samples collected from Steller sea lions during branding and capture trips between 2000-2001. This project was initiated with funding in 2000 through the Pollock Conservation Cooperative Research Center (PCCRC) and continued support will be through the ASLC. This collaborative research project between Dr. Shannon Atkinson (UAF/Alaska Sea Life Center), Matt Myers (Doctoral candidate) and Dr. Lorrie Rea (ADFG/UAF) is part of a doctoral dissertation research project undertaken by Mr. Myers and will be published in dissertation format as well as in peer-reviewed scientific journals.

Task 3G. Determining rates of metabolism in free-ranging juvenile Steller sea lions. Last year we began a new avenue of research to determine the whole animal metabolic rate of a subset of the juvenile sea lions captured during our physiology research by indirect calorimetry using open circuit respirometry (a portable metabolic chamber).

This technique has been used successfully in a captive setting both at the Vancouver Aquarium and recently at the Alaska Sea Life Center to determine how much oxygen an animal consumes over time under various environmental and physiological conditions. The results of this research have prompted authors to propose that a depressed metabolic state (lower relative oxygen utilization) would be evident in animals subject to nutritional stress. Given that our captured animals typically are left to rest quietly in capture boxes for a minimum of 1.5 hours during equilibration of deuterium for body composition measurements, this provides an excellent opportunity to collect oxygen consumption data without increasing the holding time of the animals. This equilibration period follows initial blood sampling and thus is greater than one hour after initial capture of the juveniles such that any stress of capture has been reduced.

Several factors that influence the metabolic rate of the individual will be measured or determined and used in a covariate analysis of these results, including, age of the animal, body mass, percent body fat, relative time since last meal (determined by presence of stomach contents and presence in the blood of chylomicrons or absorbed lipids from the gut, and levels of key nutritional metabolites such as ketone bodies), body temperature (rectal) and the observed behavior of the individual during the test (calm, but not sleeping). The results of these metabolic rate measurements will be used in concert with other physiological indices of condition outlined above to test the hypothesis that animals in the areas of greatest decline are currently impacted by nutritional stress. Dr. Rea has experience in measuring metabolic rate in this manner in three pinniped species (hooded seals, elephant seals and California sea lions) and our collaborators from University of Central Florida (Worthy and Hoopes) have collectively been conducting this type of research for over 15 years. During the preliminary field trials in Prince William Sound in November 2003, data was successfully collected from 23 juvenile sea lions, and we began to investigate the possible effects of anesthesia and capture stress on metabolic rate. Additional field measurements are planned during February 2004 captures in Southeast Alaska and on all future capture trips where logistically feasible.

4. Identification of diet composition; Scat analysis.

Diet analysis from fecal samples. Our research program is continuing to facilitate the annual collection of fecal samples (scat) from haulouts and rookeries visited during the brand re-sighting cruise in June-July. The scat samples have been used for prey studies undertaken by Dr. Andrew Trites at the North Pacific Marine Mammal Research Consortium and NMML. This is a continuation of previous collaborations in this area. This work will occur in each year of the grant.

5. Estimation of survival and natality rates of Alaskan Steller sea lions. In order to model the population dynamics of SSLs in Alaska and to understand what combination of age-specific survival and natality is resulting in a declining population in Western Alaska, techniques are needed to estimate survival and natality rates. It appears that the best prospect for estimating these parameters will result from branding young sea lions (Merrick *et al*. 1996) and using mark/recapture models. Estimation of vital rates through mark/resight techniques is a long-term process with a 3 to 4 year delay before initial estimates of juvenile survival can be generated and many additional years before age-specific estimates of survival and fecundity can be generated for adult age classes. This requires a long-term commitment of resources by project managers as well as funding agencies.

Task 5A. **Branding program of pups $\leq$ 2 months of age for future population dynamics analyses**. The summer of 2004 will mark the 4th consecutive year that ADFG will brand up to 300 pups at each of two rookery locations in southeast Alaska (June – July). These studies will contribute to understanding Steller sea lion population dynamics in Southeast Alaska, for comparison to similar work undertaken by the National Marine Mammal Laboratory in the Aleutian Islands, and Gulf of Alaska. Direct estimates of age-specific survival, reproduction and dispersal can be obtained through branding a large number of individuals and establishing subsequent years of re-sighting effort. Mark-Recapture analysis will be performed using models that allow for an open population. In addition, all juvenile animals (2 – 36 months of age) handled using the underwater capture technique also receive a unique permanent brand mark, for the purpose of identifying recaptured animals and to contribute to our population dynamics study.

Beginning in the first year of this proposal we anticipate that we will begin to see the first breeding females from the 2001 pup branding. This sample will increase with subsequent years and depending on sample size, an analysis of juvenile survivorship should be feasible by year three.

Task 5B. **Brand re-sighting efforts to document activities and locations of 93/94 Forrester Island cohort and pups branded in 2001 and afterward**. Annual brand re-sighting efforts were initiated in 1999 to identify and photograph sea lions that were branded on Forrester Island in 1993 and 1994, and have continued on an annual basis. During this contract period we propose to undertake a 21-day brand re-sighting cruise in southeast Alaska each year to search for any marked sea lion in the area. Additionally, Tom Gelatt serves as a Co-investigator with Dr. Andrew Trites (UBC) on a project designed to collect brand resights in British Columbia. ADFG SSL program members will work with scientists from UBC to collect those resights in late July, of each year of this proposal.

Researchers will photograph all branded animals viewed at known haulout and rookery locations in this range to confirm identification of individuals, to reassess brands that are unreadable in field conditions and to develop a catalogue of unique brand markings for the eastern population. We anticipate a significant increase in sightings of branded animals with the addition of > 600 animals branded during fieldwork in each year since 2001. This number continues to increase annually. Land-based observers will also be deployed in a field camp on Forrester Island in southeast Alaska to document the activities of branded animals and to make daily counts of rookery population during the breeding season. These ship- and land-based re-sighting efforts are required to provide the vital life history statistics to understand changes in sea lion population and are critical for the development of energetics-based population models. Incidental to this work will be the photographing and cataloging of all sea lions showing signs of entanglement. This project forms the foundation of much of our work and will continue in a consistent manner for the duration of the granting period.

Task 5C. **Creation of a brand resighting field form and training program for other researchers**. During the last funding period the ADFG SSL program created and distributed a field form and slide program to instruct other groups on brand resight collection. Additional training workshops will occur during the first two years of this grant in different coastal communities in Alaska.

Task 5D. **Investigation of post-branding pup mortality and dispersal at Lowrie Island.** In 2001 and 2002, the field crew at Lowrie Island collected resighting data on branded pups for a study of pup survival, dispersal, and tag loss. Preliminary results from this work were presented in January 2003 at the joint SSLRI/EVOS/NPRB meetings in Anchorage. The final analysis of this work will be completed in the first year of this grant.

Task 5E. Collaboration with Colorado State University (CSU) to investigate early pup survival. ADFG and NMML are co-sponsering Christopher Curgus, a graduate student at CSU to conduct his thesis research investigating the survivorship of newborn sea lions at Lowrie Island. This project is headed by Dr. Barry Noon at CSU. Chris is expected to defend his thesis during the first year of this grant. A manuscript describing the results is anticipated during the second year. Our research program will assist in the writing and supporting of the publication costs of the ms.

Task 5F. **Juvenile dependence study**. We intend to use short brand resighting trips into Prince William Sound and Southeast Alaska to allow observations of nursing juveniles on haulouts. Over time, this work will produce an estimate of the frequency of dependent juveniles in each stock and may help us understand possible differences in the demographics of the populations.

Task 5G. Database construction and continued modification. In the previous grant year we contracted with a company specializing in database construction to create a database that would incorporate all of our demographic and field data. This includes the merging of old data collected during the 1980's and never entered into a database and more recent data using a variety of collection methods. This project continues to be very productive and ongoing. The first year of this grant will be used to fund further construction of the database as the contractor works in collaboration with our scientists. Funds will be budgeted each subsequent year for the continued maintenance and upgrading of the system to meet our changing needs and the changing format of new data.

Task 5H. Age estimation technique for field captures of sea lions. During the last 3 years we have been measuring tooth lengths in live-captured sea lions for a study of ageing techniques. Using known-age animals we are still in the process of collecting a large enough sample to create a regression curve that could be used by researchers to estimate the age of a sea lion while still in the field. This is an advantage over conventional means of pulling the tooth and waiting for sectioning to estimate an age from the tooth annuli. If a researcher can estimate the age in the field they are better able to determine what samples to take or instruments to deploy depending on their questions. We will continue to collect measurements of wild-caught sea lions and by year 3 anticipate having a large enough sample to produce a peer-reviewed manuscript. Teeth will be measured during capture trips at different times of the year and analysis will occur throughout the winter. Teeth will also be pulled from some known-age animals for tooth sectioning at an out of state contractor.

6. Investigation of sea lion contaminant loads, disease screening, and pathological exams to determine sources of mortality. During each capture trip physical examinations are conducted on each sea lion captured, and samples are collected for serology screening, virology and bacteriology culture, and PCR of lesion tissue for identification of disease agents. Traditional serum chemistry profiles are analyzed at a standardized veterinary diagnostics lab (used for previous captive research and ongoing Steller sea lion work conducted by NMML) and complement our additional metabolite and haptoglobin (NMML) assays. Blubber, milk, hair and blood samples are also collected for organochloride and heavy metals contaminants analysis and blood samples are collected to investigate the immunological health of the individual. Fecal swabs and respiratory mucus samples are also inspected for the presence of parasites. These collections are ongoing from previous years and involve a number of collaborators working with our ADFG veterinarian, Dr. Kimberlee Beckmen and our contracted veterinary pathologist, Dr. Kathy Burek. The majority of samples have been collected in southeast Alaska and PWS over recent years and in the coming year our efforts will concentrate on gathering comparable samples from juveniles in the western population, particularly in the Aleutian Islands. We also propose to increase our efforts to recapture previously handled individuals to monitor changes in exposure to both disease and contaminants. These collections also allow for the continued development of a serum archive for retrospective disease screening to address the interactions between nutrition and disease state in these animals.

Task 6A. Epidemiology modeling. During the 2004 –2007 funding period we propose to intensify effort on the analyses of these previously collected data while continuing to collect samples for these health directions during all capture and branding excursions. At a recent Steller sea lion epidemiology workshop sponsored by the ASLC, it became apparent that future directions in health and disease assessment could be significantly enhanced by the integrated analysis of our existing health, disease and condition data. We propose to bring in an expert in epidemiology and disease modeling to work with Drs. Beckmen, Burek, O'Hara (UAF) and Goldstein (ASLC) through a Post-doctoral Research Fellowship at the University of Alaska, Fairbanks which would be supported in full through ADFG Cooperative Agreement funds. Much of the pathology and immunology work completed has either been published or is in press or preparation, however, these additional analyses would lead to a publication integrating several aspects of these studies and would lay the groundwork for efforts to plan future disease monitoring programs for Steller sea lions and other species at risk.

Task 6C. Post mortem examination and sampling. Post mortem exams of all sea lion carcasses available to researchers are conducted in the field and extensive samples are collected for distribution to other researchers. These exams can provide insight into the causes of death both natural and anthropogenic and is an ongoing project that will continue as long as field work is permitted and carcasses are available.

Task 6D. Investigation of the presence and potential impact of chlorinated fatty acids (CFAs) in sea lion tissues. This project compliments ongoing work on organochloride and heavy metal contaminants above with the investigation of the occurrence of chlorinated fatty acids (CFAs) in Steller sea lion tissues. CFAs are found in the food chain in areas where organochlorine chemicals have been either historically or currently used, and have been found in mussels and sea birds in the Aleutian Islands (Dutch Harbor, AK). Although CFAs do not elicit biomarker responses (such as P450), they have acute and adverse physiological effects. Exposure to and uptake of CFAs interfere with fat metabolism and thus may contribute to a marine mammal's ability to store or access body fat reserves. In the preliminary stages of this study we will quantify the frequency and extent of occurrence of CFAs in different tissue types (e.g. blubber, muscle, blood) in Steller sea lions. These initial investigations will be a collaborative effort with Drs. Alessa and Kennish from the University of Alaska, Anchorage. If CFAs are found, future research will characterize which types of CFAs are associated with specific tissue types and potentially investigate the physiological response of sea lion tissues to the presence of CFAs if appropriate cell lines are available at that time. This research on the impacts of CFAs would not involve live animals, thus would rely on being able to collaborate with researchers who have active cell lines from Steller sea lions available (e.g. Drs. Wise and Middlebrooks, whom were both previously funded through SSLRI, currently have cell lines active).

7. **Using genetic analyses to investigate stock delineation, breeding structure, and dispersal rates**. The current delineation of Steller sea lion populations into threatened and endangered stocks is based on genetic analysis. During past years the SSL program has collected tissue samples from sea lions for use in genetic analysis of primarily stock structure. In the previous funding period we contracted with Dr. John Bickham at Texas A&M University and Dr.s Greg O'Correy-Crow and Barbara Taylor at the NOAA/NMFS Southwest Fisheries Science Center to conduct intensive analyses of these samples. We will continue to collect samples and contract with these and possibly other researchers to examine questions relating to dispersal rates, breeding structure, dispersal, and reproductive success. This is ongoing work and intended to contribute information on how specific threats to the population might affect subpopulations differently depending on their distribution. ADFG will contribute tissue samples and interpretation of sampling areas and results. In year three of this proposal we intend to merge behavioral work and genetic analysis to investigate the relatedness of sea lions at a given rookery. This work is intended to investigate questions of movement patterns, gene flow, and the vulnerability of sea lions to various threats. Preliminary reports were presented to the Steller sea lion Recovery Team and at the biennial Marine Mammal Research Meeting.

8. Support of NMFS aerial survey of sea lion habitat throughtout Alaska. In previous funding cycles we have requested funds to contract an aerial survey of sea lion haulouts and rookeries throughout Alaska. In future years the NMFS will fund the survey and ADFG will help support the project with some equipment costs. This will occur annually.

9. Support of other funded research programs. Since 2001, new researchers have initiated projects investigating various hypotheses for the decline of the western stock of Steller sea lions funded by NMFS grants. We currently have over 30 researchers on our growing list of collaborators. The ADFG Steller sea lion program will continue to collaborate with as many new researchers as possible to facilitate their work and attempts to address various hypotheses relating to the decline of Steller sea lions. The amount of time and effort required for this coordination is unpredictable but significant. This includes the collection and distribution of samples, the coordination of vessel and field logistics, the incorporation of other research in ADFG permits and the participation in research coordination planning and meetings. These projects are using a greater proportion of our time as funds begin to decrease and will follow a timeline dictated by funding.

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Statement of Work 2004/2005

In this, the first year of our 4-year contract proposal we will be finalizing some of our satellite telemetry analysis from previous years, continuing our long-term programs based in animal marking and resighting, and initiating new research designed to refine our knowledge of Steller sea lion biology and habits. Resighting trips will occur in Southeast Alaska and at a reduced level in Prince William Sound. The final submission and publication of two manuscripts on juvenile diving and movement behavior will occur during this period. These papers are the culmination of a long period of work and will represent the greatest single sample of satellite telemetry data on Steller sea lions ever recorded. We will continue to capture and handle juvenile sea lions in Southeast Alaska and the Aleutian Islands. Newborn pups will be branded at two sights in Southeast Alaska and resighting surveys will occur concurrently. We will initiate a new study of hookworm infection in young of the year pups by capturing and sampling pups at age 2-3 months. In addition we will be in the first stages of a test project to examine the feasibility of designing a capture plan around the recapture of previously handled animals. This project will continue for the remainder of the grant period and if successful will provide the first data on the morphometric changes (i.e. growth rates) of free-ranging Steller sea lions. We will increase our sample size of tooth measurements for a study of field age-estimation techniques. A manuscript on post-branding pup survival will be prepared and we will continue to collect tissue samples for genetic work and support the annual aerial survey program. The sea lion capture and resight database will continue to be constructed and modified as necessary

This contract year will also see the completion of data analysis and initial publication of a 6 year study on fatty acid analysis of sea lion blubber to determine diet composition. The laboratory phase of fatty acid analysis of serum will be initiated and blubber and milk tissues will be archived for future analyses. Stable isotope analysis of whisker, milk and serum samples will continue in collaboration with USGS and the first publication from this work will be produced during this period. Body composition data will continue to be collected from each animal captured, as well as morphometric data and blood samples for nutritional status analyses (blood chemistry). Laboratory analyses will continue for these aspects of the program beginning in our new laboratory facilities in Fairbanks beginning in August 2004. Laboratory analysis of muscle biochemistry and histology will begin in our new lab facilities in September 2004 and sample collection for this project is expected to continue for the 4-year duration of this proposal. Elemental signature analysis (initiated as a pilot project last year) will continue as a collaborative project with USGS and the first publication of this work will be initiated during this 2004/2005 funding period. New research directions in nutritional biomarkers will be initiated late in the 2004/05 funding cycle and are expected to be continued throughout the duration of this proposal. Laboratory analysis of thyroid hormones should near completion during this cycle, and data analysis and manuscript preparation will begin. We will continue to collect data on resting metabolic rates of captured sea lions while at sea, and data analysis stages of this work will continue throughout the period. Contaminants and disease sample collection, laboratory analyses and data analysis will continue throughout this period. One publication is expected to be completed. An epidemiology modeling project will be initiated in September 2004 with the recruitment of a post-doctoral researcher trained in this area. A new research direction studying the prevalence of chlorinated fatty acids in sea lion tissue will begin late in this funding cycle with experimentation with analytical techniques on necropsy collection tissues. Post-mortem sampling of all carcasses found will continue throughout the 4 years of this proposal. We also expect to present data from the above projects at 2 scientific conferences during the next year; the Sea Lion Symposium in October 2004, and at comparative physiology meetings in April 2005.

Milestone Chart for Steller Sea Lion Investigations 2004/2005 NOAA Cooperative Agreement Proposal

Task	*June*	*July*	*Aug*	*Sept*	*Oct*	*Nov*	*Dec*	*Jan*	*Feb*	*March*	*April*	*May*
Field captures	X	X		X						X		X
1A – dive data analysis and manuscript submission	X	X	X									
1B- fatty acid diet analysis – sample collection				X						X		
1B – fatty acid diet analysis – laboratory analysis	X	X										
1B – fatty acid diet analysis – data analysis and publication	X	X	X		X	X	X	X	X	X	X	X
1B – fatty acid diet analysis – conference presentation					X						X	
1B – fatty acid serum analysis – laboratory analysis					X	X	X	X	X	X	X	X
1C – stable isotope sample collection	X	X		X						X		
1C – stable isotope laboratory analysis (Denver)			X	X	X					X	X	
1C – stable isotope data analysis and publication	X	X	X					X	X		X	
1C – Stable isotope analysis – conference presentation					X						X	
1E – O_2 capacity – data analysis and publication	X	X	X	X								
2A – location data final ms submission & publication	X	X	X	X							X	
2B – Satellite telemetry work collaboration						X						
3A – body composition analysis - collection				X						X		
3A – body composition analysis - lab analysis	X	X			X						X	
3A – body composition analysis - data analysis and ms prep.		X	X		X	X					X	
3A – body composition analysis – conference presentation					X						X	
3B – nutritional state – sample collection				X						X		
3B – nutritional state – laboratory analysis			X	X	X	X	X	X	X	X	X	X
3B – nutritional state – conference presentation					X						X	
3B – Juvenile captures and Hookworm Study			X									
3C – muscle biochemistry and histology – sample collection	X	X		X						X		
3C – muscle biochemistry and histology – lab analysis				X				X	X	X	X	X
3D – elemental signature analysis – sample collection	X	X		X						X		
3D – elemental signature analysis – lab analysis			X	X							X	
3D – elemental signature analysis – data analysis and pub			X	X			X	X	X			
3D – elemental signature analysis – conference presentation					X							
3E – nutritional biomarkers – sample collection				X						X		
3E – nutritional biomarkers – laboratory analysis											X	X
3F- thyroid and leptin hormones – sample collection				X						X		
3F – thyroid and leptin hormones - analysis and manuscript preparation (led by Matt Myers, UAF)	X	X	X	X	X	X	X	X	X	X	X	X
3G – rates of metabolism – data collection				X						X		
3G – rates of metabolism – data analysis and conference	X	X	X	X	X						X	

4B- scat collection		X	X										
5A – branding program – branding of young pups		X	X										
5B - brand resighting survey, sea- and land-based		X	X	X									
5C – Brand Resighting training					X							X	X
5D – post-branding pup mortality study								X					
5E – Collaboration with CSU – pup natality				X	X	X	X						
5F – Juvenile dependence study - resights	X	X	X	X			X		X		X		X
5G – Database construction and maintenance	X	X	X	X	X	X	X	X	X	X	X	X	X
5H – Age estimation techniques – collection & analysis				X		X					X		
6A – contaminants and disease – sample collection		X	X		X						X		
6A- contaminants and disease – analysis and publication			X	X	X	X	X	X	X	X	X	X	X
6B – epidemiology modeling – data analysis					X	X	X	X	X	X	X	X	X
6C – post mortem exam – sample collection		X	X		X						X		
6D – chlorinated fatty acids – sample collection		X	X		X						X		
6D – chlorinated fatty acids – laboratory analysis						X	X	X	X	X	X	X	X
7 – Genetics Analsyes		X	X										
8 – Aerial Survey (equipment & analysis support)	X	X				X							
9 – support of other SSLRI and CIFAR etc projects – sample collection		X	X		X		X				X		X
2005 NOAA Coop Agreement Proposal and budget review									X	X	X		

Statement of Work 2005-2006

In the second year of this grant we will finalize any manuscript publication related to our satellite telemetry deployments from previous years. We will also be in the analysis and writing stage of collaborative projects with the NMFS Auke Bay lab and other cooperators that have deployed instruments during our cruises. We will continue our intensive handling, branding, and sampling of newborn pups by visiting three rookeries in Southeast Alaska. Scat collection will occur during the resight cruse. The pup natality study manuscript should be ready for submissionOur study of hookworm infection rate will continue with sampling of newborn and 2-3 month –old pups. Juvenile captures will occur in the Aleutian Islands and Southeast Alaska. Our field age-estimation technique project will continue and we anticipate that by the second or third year of this grant the sample size will be sufficient to produce a robust analysis and regression curve of age based on canine length. We will continue to collect tissue samples for genetic work and support the annual aerial survey program. We will continue to collaborate with and support other Steller sea lion research when possible. The sea lion capture and resight database will be in the final stages of construction and need to be updated to accommodate the new data from the previous year.

This contract year will see the completion of the second publication from our fatty acid analysis of sea lion diet composition. The laboratory phase of fatty acid analysis of serum will continue and blubber and milk tissues will be archived for future analyses. Stable isotope analysis of whisker, milk and serum samples will continue in collaboration with USGS. Body composition data will continue to be collected from each animal captured, as well as morphometric data and blood samples for nutritional status analyses (blood chemistry). Laboratory analyses will continue for these aspects of the program. Laboratory analysis of muscle biochemistry and histology will continue during this period and sample collection for this project is expected to continue for the 4-year duration of this proposal. Elemental signature analysis will continue as a collaborative project with USGS and the first publication of this work will be completed during this funding period. New research directions in nutritional biomarkers will continue from the 2004/05 funding cycle and are expected to be continued throughout the duration of this proposal. Data analysis of thyroid hormones should near completion during this cycle, and dissertation and manuscript preparation should also be completed.

We will continue to collect data on resting metabolic rates of captured sea lions while at sea, and data analysis stages of this work will continue throughout the period. Contaminants and disease sample collection, laboratory analyses and data analysis will continue throughout this period at a slower rate to facilitate financial support of the epidemiology position. The epidemiology modeling project will be continued throughout this period with data analysis playing the largest role. New research into chlorinated fatty acids in sea lions should have sample analysis underway and enter into data analysis late in the funding cycle. Post-mortem sampling of all carcasses found will continue throughout the 4 years of this proposal. We also expect to present data from the above projects at 2 scientific conference during the next year; the International conference on the Biology of Marine Mammals in December 2005, and at comparative physiology meetings in April 2006.

Milestone Chart for Steller Sea Lion Investigations 2005/2006 NOAA Cooperative Agreement Proposal

Task	*June*	*July*	*Aug*	*Sept*	*Oct*	*Nov*	*Dec*	*Jan*	*Feb*	*March*	*April*	*May*
Field captures	X	X		X						X		
1A – dive data analysis and manuscript submission	X	X	X	X	X	X	X					
1B- fatty acid diet analysis – sample collection				X						X		
1B – fatty acid diet analysis – laboratory analysis						X	X					
1B – fatty acid diet analysis – data analysis and publication	X	X	X				X					
1B – fatty acid serum analysis – laboratory analysis	X	X	X	X	X	X	X	X			X	
1B – fatty acid serum analysis – data analysis							X	X	X	X	X	X
1C – stable isotope sample collection	X	X		X						X		
1C – stable isotope laboratory analysis (Denver)			X	X	X					X	X	
1C – stable isotope data analysis and publication	X	X	X					X	X		X	X
1C – Stable isotope analysis – conference presentation							X				X	
2A – location data analysis and manuscript submission	X	X	X	X	X	X	X					
2B – Satellite telemetry work collaboration							X					
3A – body composition analysis - collection				X						X		
3A – body composition analysis - lab analysis	X	X			X						X	
3A – body composition analysis - data analysis and ms prep.		X	X		X	X					X	X
3A – body composition analysis – conference presentation							X				X	
3B – nutritional state – sample collection				X						X		
3B – nutritional state – laboratory analysis	X	X	X	X	X	X	X	X	X	X	X	X
3B – nutritional state – conference presentation							X				X	
3C – muscle biochemistry and histology – sample collection	X	X		X						X		
3C – muscle biochemistry and histology – lab analysis			X	X	X	X	X	X	X	X	X	X
3D – elemental signature analysis – sample collection	X	X		X						X		
3D – elemental signature analysis – lab analysis			X	X							X	
3D – elemental signature analysis – data analysis and pub			X	X			X	X	X			
3D – elemental signature analysis – conference presentation							X				X	
3E – nutritional biomarkers – sample collection	X	X		X						X		
3E – nutritional biomarkers – laboratory analysis			X	X	X	X	X	X	X	X	X	X
3F – thyroid and leptin hormones - analysis and manuscript preparation (led by Matt Myers, UAF)	X	X	X	X	X	X	X					
3G – rates of metabolism – data collection				X						X		X
3G – rates of metabolism – data analysis and conference	X	X	X	X	X		X				X	
4B- scat collection	X	X		X		X				X		
5A – branding program – branding of young pups	X	X										

5B - brand resighting survey, sea- and land-based	X	X	X									
5C – Brand Resighting training					X							X
5D – post-branding pup mortality study	X	X	X	X	X							
5E – Collaboration with CSU – pup natality				X	X	X	X					
5F – Juvenile dependence study - resights	X	X	X	X			X		X		X	
5G – Database construction and maintenance	X	X	X	X	X	X	X	X	X	X	X	X
5H – Age estimation techniques – collection & analysis				X		X					X	
6A – contaminants and disease – sample collection	X	X		X						X		
6A- contaminants and disease – analysis and publication		X	X	X	X	X	X	X	X	X	X	X
6B – epidemiology modeling – data analysis	X	X	X	X	X	X	X	X				
6B – epidemiology modeling – conference and publication							X	X	X	X		
6C – post mortem exam – sample collection	X	X		X						X		
6D – chlorinated fatty acids – sample collection	X	X		X						X		
6D – chlorinated fatty acids – laboratory analysis	X	X	X	X	X	X	X	X	X	X	X	X
6D – chlorinated fatty acids – data analysis and coference	X	X	X	X	X	X	X					
7 – Genetics Analsyes		X	X									
8 – Aerial Survey (equipment & analysis support)	X	X				X						
9 – support of other SSLRI and CIFAR etc projects – sample collection		X	X		X		X				X	
2006 NOAA Coop Agreement Proposal and budget review								X	X	X		

Statement of Work 2006-2007

In the third year of this grant we be focusing heavily on conducting a survivorship analysis of branded sea lions based on the animals handled since 2001. This analysis will be the most intensive investigation of sea lion survival to date. We will continue our intensive handling, branding, and sampling of newborn pups by visiting two rookeries in Southeast Alaska. Scat collection will occur during the resight cruse. Our study of hookworm infection rate will continue with sampling of newborn and 2-3 month –old pups. Juvenile captures will occur in the Aleutian Islands and Southeast Alaska. Our field age-estimation technique project will continue and we anticipate that a manuscript will be in preparation on the analysis and regression curve of age based on canine length. We will continue to collect tissue samples for genetic work and support the annual aerial survey program. We will continue to collaborate with and support other Steller sea lion research when possible. A report on the juvenile dependence study will be in preparation. The sea lion capture and resight database will continue to be updated and modified as necessary.

During this contract year laboratory phase of fatty acid analysis of serum will continue and data analysis will begin. Blubber and milk tissues will continue to be archived for future fatty acid analyses. Stable isotope analysis of whisker, milk and serum samples will continue in collaboration with USGS. Body composition data will continue to be collected from each animal captured, as well as morphometric data and blood samples for nutritional status analyses (blood chemistry). Laboratory analyses will continue for these aspects of the program and we expect one publication pertaining to this research to be submitted during this period. Laboratory analysis of muscle biochemistry and histology will continue during this period and sample collection for this project is expected to continue for the 4-year duration of this proposal. Elemental signature analysis of whiskers will continue as a collaborative project with USGS and related work on the potential impact of low micronutrients on the condition of other tissues will be initiated during this funding period. Laboratory and data analysis on nutritional biomarkers will continue throughout the duration of this proposal. We will continue to collect data on resting metabolic rates of captured sea lions while at sea, and data analysis and manuscript/dissertation preparation of this work will continue throughout the period. Contaminants and disease sample collection, laboratory analyses and data analysis will continue throughout this period. The epidemiology modeling project should be completed during this period with data analysis and manuscript preparation playing the largest role. Research into chlorinated fatty acids will be in the data analysis stage during this funding cycle. Post-mortem sampling of all carcasses found will continue throughout the 4 years of this proposal. We also expect to present data from the above projects at 2 scientific conferences during this funding cycle.

Milestone Chart for Steller Sea Lion Investigations 2006/2007 NOAA Cooperative Agreement Proposal

Task	*June*	*July*	*Aug*	*Sept*	*Oct*	*Nov*	*Dec*	*Jan*	*Feb*	*March*	*April*	*May*
Field captures	X	X		X						X		
1A – dive data analysis and manuscript submission	X	X	X	X	X	X	X					
1B- fatty acid diet analysis – sample collection				X						X		
1B – fatty acid diet analysis – conference presentation											X	
1B – fatty acid serum analysis – laboratory analysis	X	X	X	X	X	X	X	X	X	X	X	X
1C – stable isotope sample collection	X	X		X						X		
1C – stable isotope laboratory analysis (Denver)			X	X	X					X	X	
1C – stable isotope data analysis and publication	X	X	X					X	X		X	X
1C – Stable isotope analysis – conference presentation					X						X	
2A – location data analysis and manuscript submission												
2B – Satellite telemetry work collaboration							X					
3A – body composition analysis - collection				X						X		
3A – body composition analysis - lab analysis	X	X			X	X					X	X
3A – body composition analysis - data analysis and ms prep.		X	X		X	X	X				X	X
3A – body composition analysis – conference presentation					X						X	
3B – nutritional state – sample collection				X						X		
3B – nutritional state – laboratory analysis	X	X	X	X	X	X	X	X	X	X	X	X
3B – nutritional state – conference presentation					X						X	
3B – Juvenile captures				X								
3C – muscle biochemistry and histology – sample collection	X	X		X						X		
3C – muscle biochemistry and histology – lab analysis					X	X	X	X	X		X	X
3D – elemental signature analysis – sample collection	X	X		X						X		
3D – elemental signature analysis – lab analysis			X	X							X	
3D – elemental signature analysis – data analysis and pub				X	X		X	X	X			X
3D – elemental signature analysis – conference presentation					X						X	
3E – nutritional biomarkers – sample collection				X						X		
3E – nutritional biomarkers – laboratory analysis	X	X	X			X	X				X	X
3G – rates of metabolism – data collection				X						X		
3G – rates of metabolism – data analysis and thesis prep.	X	X	X	X	X	X					X	
4B- scat collection	X	X		X		X				X		
5A – branding program – branding of young pups	X	X										
5B - brand resighting survey, sea- and land-based	X	X	X									
5C – Brand Resighting training					X							X
5F – Juvenile dependence study - resights	X	X	X	X			X		X		X	
5G – Database maintenance	X	X	X	X	X	X	X	X	X	X	X	X

5H – Age estimation techniques – collection & analysis				X		X					X	
6A – contaminants and disease – sample collection	X	X		X						X		
6A- contaminants and disease – analysis and publication		X	X		X	X	X	X	X		X	X
6B – epidemiology modeling – publication	X	X	X	X								
6C – post mortem exam – sample collection	X	X		X						X		
6D – chlorinated fatty acids – sample collection	X	X		X						X		
6D – chlorinated fatty acids – laboratory and data analysis	X	X	X	X	X	X	X	X	X	X	X	X
7 – Genetics Analsyes		X	X									
8 – Aerial Survey (equipment & analysis support)	X	X				X						
9 – support of other SSLRI and CIFAR etc projects – sample collection		X	X		X		X				X	
2007 NOAA Coop Agreement Proposal and budget review								X	X	X		

Statement of Work 2007-2008

In the final year of this grant we will be continuing to focus on a survivorship analysis of branded sea lions based on the animals handled since 2001. We will also continue to mark newborn pups and conduct resighting surveys throughout Southeast Alaska and Prince William Sound. We will collaborate with other researchers collecting resights and continue our instruction on methods. We will continue our intensive handling, branding, and sampling of newborn pups by visiting three rookeries in Southeast Alaska. Scat collection will occur during the resight cruse. Our study of hookworm infection rate will be in its final stages and manuscript preparation will begin. Juvenile captures will occur in the Aleutian Islands and Southeast Alaska. We will continue to collect measurements for our field age-estimation technique project to increase the existing sample size. We will continue to collect tissue samples for genetic work and support the annual aerial survey program. We will continue to collaborate with and support other Steller sea lion research when possible. A final report on the juvenile dependence study will available. The sea lion capture and resight database will continue to be updated and modified as necessary.

During this contract year laboratory phase of fatty acid analysis of serum will be completed and data analysis and thesis/manuscript preparation will be the main focus of this task. Blubber, milk and additional serum tissues will continue to be archived for future fatty acid analyses. Stable isotope analysis of whisker, milk and serum samples will continue in collaboration with USGS. Body composition data will continue to be collected from each animal captured, as well as morphometric data and blood samples for nutritional status analyses (blood chemistry). Laboratory analyses will continue for these aspects of the program. Laboratory analysis of muscle biochemistry and histology will continue during this period and sample collection for this project is expected to continue for the 4-year duration of this proposal. This project should also enter data analysis and dissertation/manuscript preparation later in this funding cycle. Elemental signature analysis of whiskers will continue as a collaborative project with USGS and related work on the potential impact of low micronutrients on the condition of other tissues will be continued during this funding period. Laboratory and data analysis on nutritional biomarkers will continue throughout the duration of this proposal. We will continue to collect data on resting metabolic rates of captured sea lions while at sea, and data analysis and manuscript/dissertation preparation of this work should be completed during this period. Contaminants and disease sample collection, laboratory analyses and data analysis will continue throughout this period. The epidemiology modeling project should in the final stages of publication during this period. Research into chlorinated fatty acids will be in the final stages of data analysis during this funding cycle with thesis and manuscript preparation underway. Post-mortem sampling of all carcasses found will continue throughout the 4 years of this proposal. We also expect to present data from the above projects at 2 scientific conferences during the next year.

Milestone Chart for Steller Sea Lion Investigations 2007/2008 NOAA Cooperative Agreement Proposal

Task	*June*	*July*	*Aug*	*Sept*	*Oct*	*Nov*	*Dec*	*Jan*	*Feb*	*March*	*April*	*May*
Field captures	X	X		X						X		X
1A – dive data analysis and manuscript submission												
1B- fatty acid diet analysis – sample collection				X						X		
1B – fatty acid serum analysis – laboratory and data analysis	X	X	X	X	X	X	X					
1B – fatty acid serum analysis – thesis prep and conference						X	X	X	X	X	X	X
1C – stable isotope sample collection	X	X		X						X		
1C – stable isotope laboratory analysis (Denver)			X	X	X					X	X	
1C – stable isotope data analysis and publication	X	X	X					X	X		X	
1C – Stable isotope analysis – conference presentation					X						X	
2A – location data analysis and manuscript submission	X	X	X	X	X	X	X					
2B – Satellite telemetry work collaboration							X					
3A – body composition analysis - collection				X						X		
3A – body composition analysis - lab analysis					X	X					X	X
3A – body composition analysis - data analysis and ms prep.		X	X		X	X						
3A – body composition analysis – conference presentation					X						X	
3B – nutritional state – sample collection				X						X		
3B – nutritional state – laboratory analysis			X	X	X	X	X	X	X	X	X	X
3B – nutritional state – conference presentation					X						X	
3C – muscle biochemistry and histology – sample collection	X	X		X						X		
3C – muscle biochemistry– lab and data analysis	X	X	X		X	X	X	X	X			
3C – muscle biochemistry – dissertation/manuscript prep										X	X	X
3D – elemental signature analysis – sample collection	X	X		X						X		
3D – elemental signature analysis – lab analysis			X	X							X	
3D – elemental signature analysis – data analysis and pub	X	X	X		X	X	X	X	X			
3D – elemental signature analysis – conference presentation					X							
3E – nutritional biomarkers – sample collection				X						X		
3E – nutritional biomarkers – laboratory analysis	X	X	X		X	X	X	X	X		X	X
3G – rates of metabolism – data collection				X						X		
3G – rates of metabolism – data analysis and thesis/ms prep.	X	X	X	X	X	X					X	
4B- scat collection	X	X		X		X				X		X
5A – branding program – branding of young pups	X	X										
5B - brand resighting survey, sea- and land-based	X	X	X									
5C – Brand Resighting training					X							X
5F – Juvenile dependence study - resights							X		X		X	
5G – Database construction and maintenance	X	X	X	X	X	X	X	X	X	X	X	X

6A – contaminants and disease – sample collection	X	X		X						X		
6A- contaminants and disease – analysis and publication		X	X	X	X	X	X	X	X	X	X	X
6C – post mortem exam – sample collection	X	X		X						X		
6D – chlorinated fatty acids – sample collection	X	X		X						X		
6D – Chlorinated fatty acids – thesis and manuscript prep									X	X	X	X
7 – Genetics Analsyes		X	X									
8 – Aerial Survey (equipment & analysis support)	X	X				X						
9 – support of other SSLRI and CIFAR etc projects – sample collection		X	X		X		X				X	
2008 NOAA Coop Agreement Proposal preparation								X	X	X		