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

II. Date of application: December 1, 2006

III. Applicant and Personnel

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B. Qualifications/experience of PI and CIs*

*See attached qualifications (CVs).

IV. PROPOSAL

A. Summary

This permit application covers research to be performed on free ranging and temporarily captive Steller sea lions, *Eumetopias jubatus*, by the Alaska SeaLife Center (ASLC) from June 2007-2012. The purpose of this research is to investigate causes for the Steller sea lion population decline and determine what is currently limiting its recovery.Individuals may be taken by the following means with maximum number of takes per year in parentheses: disturbance associated with capture, remote video studies, scat collection and mark resighting (14,000), capture, restraint and sampling (610) and temporary captivity (30). Captured sea lions will undergo morphometrics measurement, blood and tissue collection, digital imaging, hot-branding, scientific instrument attachment, body condition measurement, whisker sampling, metabolic rate measurement, temporary marking, x-ray exams and life history transmitter implantation (30). Individuals will be taken from the Gulf of Alaska and the Aleutian Islands. Projects will focus on population monitoring, health, nutrition and foraging behavior and will provide a wide range of information, including data on pup and juvenile survival, reproductive rates, diet, epidemiology, endocrinology, immunology, virology, physiology, ontogenetic and annual body condition cycles, foraging behavior and habitat selection. No non-target marine mammals or species listed under the Endangered Species Act are expected to be taken.

B. Introduction

1. Species

The target species to be taken is the Steller sea lion, *Eumetopias jubatus*, specifically the western stock, distinct population segment (DPS) from Cape Suckling (144º W) in the northern Gulf of Alaska through the Aleutian Islands. No non-target species will be affected by our activities. The Western DPS of Steller sea lions is currently listed as "endangered" under the Endangered Species Act (ESA) and "depleted" under the Marine Mammal Protection Act (MMPA). The minimum abundance estimate for the Western DPS was 38,513 animals in 2004 which is a 6.3% increase since 2002 but still 32.6% below 1990 levels and 81.3% below peak abundance in the late 1970s (NMFS Stock Assessment Report 2005).

Non-target avian species that may be incidentally disturbed by our research include: fork-tailed storm-petrel (*Oceanodrama furcata*), double-crested cormorant (*Phalacrocorax auritus*), pelagic cormorant (*Phalacrocorax pelagicus*), red-faced cormorant (*Phalacrocorax urile*), glaucous-winged gull (*Larus glaucescens*), black-legged kittiwake (*Rissa tridactyla*), common murre (*Uria aalge*), thick-billed murre (*Uria lomvia*), pigeon guillemot (*Cepphus columba*), parakeet auklet (*Aethia psittacula*), tufted puffin (*Fratercula cirrhata*) and horned puffin (*Fratercula corniculata*). None of those species are listed under the ESA or Convention on International Trade in Endangered Species.

2. Background/Literature Review

The following sections are divided into two major ASLC projects (Tasks) that focus on different approaches to studying Steller sea lion biology. **Task 1** focuses on free-ranging Steller sea lions and includes instrumentation of sea lions with telemetry devices to study behavior and physiology, blood and tissue sample collection, remote video monitoring, scat and opportunistic carcass collection, pup marking and mark resighting. **Task 2** includes the Transient Juvenile Program and health assessment of free-ranging juveniles.

Task 1

There are undoubtedly a large number of possible causes of the Steller sea lion population decline, but there are only three ultimate mechanisms to consider: 1) a decline in adult or juvenile survival, 2) a decline in fecundity, or 3) an increase in emigration out of the Western DPS. Emigration is an unlikely explanation because range-wide surveys conducted since 1989 have failed to find large numbers of "missing" sea lions (Loughlin 1998). On the other hand, decreased fecundity may have played an important role in both the early decline and in the current situation. Modeling studies have suggested that a 20-30% drop in juvenile survival was the most likely determinant of the steep population decline in the 1980's (York 1994), but in the last five years, juvenile survival has returned to pre-decline levels (Holmes and York 2003). Reproductive rates were also apparently low during the 1980's, and they have remained low throughout the 1990's and 2000's, suggesting that poor reproductive performance might be the most important factor currently limiting the recovery of the Western DPS (Holmes et al. 2005).

Low fecundity could be due to many different factors, such as disease, contaminants, parasitic loads, or nutritional stress. A great deal of research has focused on the possible role that nutritional stress played in the decline of Steller sea lions because of the lack of evidence for other single cause hypotheses (DeMaster and Atkinson, 2002). The main evidence that Steller sea lions in the Western stock were nutritionally stressed during the period that their population was declining comes from a comparison of female sea lions collected in 1975–1978 with females collected in 1985–1986 (Calkins and Goodwin, 1988). The early collection coincided with the approximate start of the decline, and the later collection occurred during a time when the population was declining sharply. The comparison showed that in the 1980's females were shorter, thinner, and had lower masses, such that overall body growth was reduced compared with the 1970's (Calkins et al. 1998). Additional support for the nutritional stress hypothesis was that late gestation pregnancy rates fell from 67% in the 1970's to only 55% in the 1980's (Pitcher et al. 1998). Although this difference was not statistically significant, it is possible that the population decline was already underway at the time the first collections were made (Calkins et al. 1998), so perhaps the surprisingly low pregnancy rate from the 1970's was depressed below a previously unstressed level.

The main objective of Task 1 is to continue to gather baseline data on the behavior, food habits and health and physiology of Steller sea lions with a specific emphasis on aspects related to reproductive performance. Although much of our work will focus on maternal behavior and physiology, we will also assess other aspects of Steller sea lion biology taking a holistic approach to understanding the factors that might be limiting the recovery of Steller sea lion populations. One of our goals is to determine how foraging

success relates to reproductive success. One of the key data gaps pointed out in the draft Steller sea lion Recovery Plan (NMFS 2006) is that the mechanism by which changes in fish populations affect marine mammal populations is currently unknown. We plan to address this critical information gap by monitoring the behavior of adult female Steller sea lions at sea and on land and exploiting temporal and spatial variation in prey bases and population trends. Because low juvenile survival was a problem in the past and because low fecundity may result from inadequate maternal provisioning during developmental stages, we will also study pups from birth through sexual maturation. Short-term studies will collect extremely high-resolution data on the behavioral and physiological ecology of individual sea lions in geographic areas that are undergoing concurrent fish stock assessment. These projects should provide valuable information on the foraging tactics of Steller sea lions and how they might be affected by changes in prey type or availability. By conducting long-term studies on individuals in areas of varying population trends, we hope to gain insight into the demographic consequences of differences in behavioral strategies and physiological condition.

Foraging Ecology

The main aim of studying Steller sea lion foraging is to assess how variation in prey type, abundance and distribution affect the behavior, condition, survival and reproductive success of individual sea lions. We will attempt both short-term (1–2 weeks) and long-term telemetry deployments in order to achieve our goals. For the short-term studies, we will deploy instruments that collect very high-resolution data on the movements and behavior of Steller sea lions. These include archival tags that will have to be retrieved from the sea lions at the completion of the study. The tags will record many parameters, including dive depth, swim speed, body orientation and acceleration, flipper movements, prey ingestion, images of prey acquisition, physiological parameters such as heart rate and breathing frequency and geographic position. We will capture Steller sea lions at various locations in the Gulf of Alaska and the Aleutian Islands. Upon initial capture, the sea lions will be anesthetized for instrument attachment and biological sampling. Blood and tissue samples will be obtained for assessing health and physiological condition, and samples will also be supplied to other projects. Body condition will be assessed using labeled water dilution, bioelectrical impedance and 3D photogrammetry. Whenever possible, sea lions will be monitored when spatially and temporally concurrent fish stock assessments are being conducted. High-resolution data from these short-term studies should complement studies done by us and others that collect data on foraging behavior and movements for a longer period of time but at a poorer resolution. We will distribute these data and work collaboratively with other groups that are undertaking modeling studies of the behavior and energetics of sea lions (D. Thompson and I. Boyd et al., Sea Mammal Research Unit, UK; S. Hinckley et al., Alaska Fisheries Science Center, US).

A key goal of the Alaska SeaLife Center remote video monitoring project is to gather data on important parameters of reproduction, such as maternal investment. Now that we have six years of video monitoring of largely undisturbed sea lions at Chiswell Island, we propose to complement this work with observations of the foraging behavior and energetics of juvenile and adult female Steller sea lions while they are at sea. Additionally, we will more directly measure the consequences of different behaviors by directly measuring maternal investment through milk intake and pup growth. The fine-

scale measurement of foraging behavior and energetics that we are proposing has been recommended by multiple Steller Sea Lion Research Peer Review Panels and was identified as an important research area by the recent National Research Council (NRC) (2003) review of the Steller sea lion population decline. The development of techniques for directly measuring prey ingestion has been led by one of our scientists (Andrews 1998), and the NRC review suggested that the application of such techniques will be important for properly characterizing the habitat needs of Steller sea lions.

The research proposed here will produce data that will make an important contribution towards the recovery of Steller sea lions. These studies respond to multiple recommendations of the Steller sea lion Recovery Plan (NMFS 2006) and focus on action recommendation #2.3 to "insure adequate habitat and range for recovery." Our research addresses multiple sub-headings of recovery action plan #2.3, such as examining diet through scat and stomach collections (2.3.1) and fatty acid analyses (2.3.2), deploying instruments to obtain fine-scale data on foraging habitat (2.3.3) and determining foraging needs (2.3.4). We will also follow the Recovery Plan recommendations #2.4 and 2.5 to determine the environmental factors influencing Steller sea lion foraging and survival and to investigate sea lion bioenergetics. The main goal of our foraging ecology research is to address Recovery Plan recommendation #2.6.5--assess the response of sea lions to changes in prey distribution and availability. Although the current Recovery Plan is still in draft form (NMFS 2006), it should be clear that the research that we are requesting permission to conduct will fulfill some very important unmet research needs that were also identified in the previous Recovery Plan (NMFS 1992) and are still considered critical to this day. This is made clear by the following quote from the Alaska Groundfish Fisheries Draft Programmatic Supplemental Environmental Impact Statement (NMFS Sept. 2003): "The largest information gaps in understanding what has caused the decline of sea lions or preventing their recovery are in the area of nutritional stress. In particular, they involve the following issues: measuring nutritional stress in a random sample of the population; determining prey and prey field requirements to sustain healthy individual sea lions; understanding sea lion use of habitat and how this changes with age and season; discerning natural from fishery-induced changes in the prey field." Our research will directly examine the nutritional and prey field needs of Steller sea lions, and it will also determine how variation, in space and in time, in the prey field affects the health and reproductive success of Steller sea lions. Therefore, this research will contribute in a very significant way to conservation efforts.

We plan to capture adult females and their pups during the peak breeding season as well as at any other time of year when a female is lactating and may be pregnant. The first two months of a pup's life can be a critical time, and it is thus important to understand the constraints on a mother's ability to provision for her offspring during this time. Two of the investigators for this project (Andrews and Calkins) have conducted previous studies on adult females and their pups during the first two months of pupping, and they have not observed any evidence that capture and handling lead to a significant increase in the rate of abandonment, pup mortality, or other signs of adverse effects on pups (Andrews et al. 2002; Brandon et al. 2005; Adams 2000).

A unique and critical advantage to our proposal is that at one of the sites that we are proposing to conduct the adult female and pup research, we will be able to directly quantify the effects of human disturbance in general and our research specifically. At

Chiswell Island, we have been monitoring the time-activity budgets and general behaviors of Steller sea lions during the last six breeding seasons using remote-video monitoring (e.g., Maniscalco et al. 2006). This study has focused on maternal investment and pup survival, but when we commence this new research, we will gather additional data so that we can better assess the effects of research on the sea lions. We will also continue to collect data in the exact same manner as in previous years so we can compare a breeding season with new research activity to the past six years of data when the rookery was relatively undisturbed. Therefore, our ability to monitor mother pup pairs on Chiswell with around the clock video observations will permit us to quantify the effect of the disturbance caused by capturing sea lions during the breeding season. We may also install a microwave-linked system at Outer Island, on the Kenai Peninsula, so we can monitor instrumented females and pups in these additional locations. The ability to directly measure the effects of human disturbance and research is an important unmet need given the intensity of Steller sea lion research being conducted currently.

We plan to recapture adult females and juveniles twice during a year and to recapture pups as many as four times annually (no more than once per week). Recapture will permit us to more precisely determine performance by measuring changes in body condition and other health parameters. By recapturing individuals at different times of the year, we will also be able to compare the foraging behavior and energetics of animals in relation to the environmental variation that will occur over space and time. On capture and recapture events, sea lions (adults and juveniles) will be weighed to examine mass changes and blubber, fecal, skin and mucosal samples. Blood samples will be taken to examine health status, and gastric lavage and enemas will be performed to examine diet. Body condition will be assessed with labeled water turnover and bioelectric impedance analysis.

During the summer breeding season, we propose to capture adult females and their pups for assessment of health status and for instrument attachment to monitor fine scale at-sea foraging behavior and energetics. Adult females will be captured on the rookery by darting with Telazol® or noosing them when they are on land or in water close to shore. Subsequent immobilization will be facilitated by anesthesia with isoflurane via intubation. Measurements of mass and other morphometrics will be made on the adult females and milk samples. Blood and blubber samples will be taken for health and body condition assessment. Adult females will also have a tooth extracted for aging and will be hot branded for long term identification.

We will attach instruments for remote monitoring of at-sea foraging behavior and energetics by gluing them to the fur. These instruments will include satellite-linked time depth recorders and archival data loggers for measuring prey ingestion and additional behavioral and physiological variables such as heart rate and flipper stroke frequency. Some females will be instrumented with an underwater animal-borne camera. As the adult females are recovering from anesthesia they will be reunited with their pups and subsequently observed with remote video and telemetry. After a measurement period of approximately 4 to 40 days, adult females will be recaptured to repeat mass and other morphometric measurements. It should be noted that for the majority of adult female Steller sea lions, the normal state is to be lactating and pregnant almost year round, year after year. Pitcher and Calkins (1981) reported that between 70% and 100% of Steller sea lion females > 6 years old are pregnant in the fall each year, with approximately 60%

giving birth successfully, and the best evidence is that young of the year are weaned near the time the mother gives birth to the next year's pup (NMFS 2006). Therefore, up to 60% of females are lactating at any given time, and the long period of lactation means that the energetic demands of lactation will vary as the pup's size and metabolic demands increase. In other pinnipeds, where lactation may be condensed into a small fraction of a year, the summer breeding season may in fact be the most energetically demanding time for a mother, but in Steller sea lions this is not the case. Therefore, when we work with adult females during the summer months, we will be working when the energetic demands of the small pup are the lowest and therefore during one of the least energetically demanding periods of an adult female sea lion's life (Winship et al. 2002).

We are proposing to capture adult females and their pups early in the breeding season (captured mother/pup pairs with pups > 5 days of age) for a number of important scientific reasons as well. For the first month of life, pups are reluctant to enter the water; therefore, the cows are highly motivated to return to the rookery after each foraging trip. Also, because foraging trips during the first month of pupping tend to be shorter than later in the season (Maniscalco et al. 2006), it appears that females are more highly motivated to stay with their pups early on. This suggests that the incidental disturbance during capture events is less likely to result in pup abandonments by other females than it would later in the season. At Chiswell Island, adult females and their pups tend to haulout further from the water early in the season. Near the end of July (when pups are about 4–6 weeks of age), the females begin occupying spots much closer to the water's edge or on other nearby offshore rocks. This behavior would make capture of adult females much more difficult later in the breeding season than it is early in the breeding season. During the peak breeding season (June and July), adult females behave as stereotypical central place foragers, with foraging trips that last only a short period of time (about 12 hours) and cover a small distance because their pups cannot survive without their mother's milk for very long. This allows us to correlate prey availability with female foraging performance on the small scales that are important to sea lions and that we can accurately measure with fishery survey techniques in a short time window. Later in the breeding season, foraging trips become longer, and the females cover larger distances. Therefore, the best time to compare foraging behavior and prey availability on a fine-scale is early in the breeding season. Also, the tendency to start moving pups away from the rookery in August might compromise our ability to collect the data that we need because we would not be able to easily find the instrumented sea lions and remotely-release the archival data-logging devices. Because it is probable that we will lose data in this way, our initial sample size will have to be higher than it would if females were captured earlier in the season when the chance of instrument and data recovery is higher.

We also propose to capture and instrument adult females at other times of the year, in order to determine how they are affected by seasonal changes in water temperature, prey availability and demands of the pup. Although later in the year is a more energetically demanding time for the female (because pup growth increases the demands on the mother), neglecting to study lactating Steller sea lions would handicap our efforts to provide knowledge that can be used to aid the recovery of Steller sea lions. A few other otariids such as Australian sea lions also have extended lactation periods, and there is no evidence that capturing adult females or their dependent young compromises either of them (Costa and Gales 2003; Fowler et al. 2006).

In addition to collecting basic data on the behavior and energetics of foraging, we also aim to determine how variations in the prey field can affect Steller sea lions. In order to do this, we will rely on two approaches: examination of foraging behavior in areas of varying prey field and experimental manipulations. We cannot control the prey field that is available to Steller sea lions, but it is clear that experimental manipulations are an extremely powerful way to obtain answers to these sorts of questions. An alternative to experimentally reducing prey density or changing the prey field composition to one with lower food quality is to experimentally increase the cost of foraging by increasing the hydrodynamic drag. This is a feasible manipulation that can be done for a very brief period of time, but this can provide valuable information on the ability of Steller sea lions to compensate for additional costs of foraging, such as might occur if prey density or quality is reduced. In order to accomplish this, a device to alter the buoyancy and drag will be temporarily attached to sea lions. In addition to altering the cost/benefit ratio of foraging, this will allow us to experimentally determine the effects of changes in body condition. Increases or decreases in the amount of body fat will affect the buoyancy of sea lions and therefore their foraging costs. There will be three different treatment groups: increased buoyancy, decreased buoyancy and a control group. Increases in buoyancy will be made by adding a block of syntactic foam and buoyancy will be decreased using a block that contains lead weights. All blocks will have the same surface area. Control blocks that are neutrally buoyant will allow us to determine the effect of the added drag--another factor that will affect the cost of foraging. These buoyancy/drag blocks will be left on for no more than four weeks. At the end of the measurement period, the archival instruments and the buoyancy/drag blocks will be removed with remote-release devices.

An experimental manipulation that can be properly controlled and replicated is the only way that we will be able to meet many goals of the Steller sea lion Recovery Plan. This was emphasized in many of the Peer Reviews of the Steller sea lion research plans, as well as in Bowen et al. (2001; Review of the November 2000 Biological Opinion and Incidental Take Statement with Respect to the Western Stock of the Steller Sea Lion, with Comments on the Draft August 2001 Biological Opinion). The Bowen report recommended experimental manipulations of fishing effort in Steller sea lion habitat in order to examine the effects of such stress on sea lions, but NMFS has determined that such experiments are impractical. A reasonable alternative, however, is to experimentally stress individual sea lions by changing the cost of foraging, which we can achieve by altering buoyancy or hydrodynamic drag. Such an increase in foraging costs can be compared with the increased cost that might be incurred by sea lions if prey density was reduced due to fishing effort or climate change. It has been suggested that some pinnipeds are routinely operating well within their physiological limits and may easily be able to cope with environmental stress by working a little harder or spending more time at sea searching for fish (Costa et al. 2001). However, some species of otariids seem to be working near their physiological limits, which may constrain their ability to respond to environmental change.

Antarctic fur seals are one of the species that appear to have a large reserve capacity (Boyd et al. 1997). In a study that fitted lactating Antarctic fur seal females with similarly proportioned drag devices, characteristics of individual dives were affected and foraging trip length increased by 10% but maternal mass and pup growth were not

different between control and experimental groups (Boyd et al. 1997). Based on the results of the study on Antarctic fur seals, in Steller sea lions, we expect to see very slight changes in parameters such as swim speed or dive descent rate, but sea lions will not be encumbered in a way that should affect their annual growth or survival rates. We also anticipate that the slight changes in drag and/or buoyancy will not significantly affect predator escape responses of Steller sea lions. During observations of captive sea lions swimming with similar drag devices, we have not seen any significant reductions in burst swim speed, maneuverability, or other factors that may be important in predator avoidance. Given what was observed in the Antarctic fur seal study, we do not anticipate any significant adverse, long-term effects on female body condition, ability to provision young, or survival. We are attempting to simulate mild changes in body condition or work load for a very limited period of time. The results should help us to predict how Steller sea lions will cope with short-term environmental fluctuations, such as increased work to find fish or physiological changes such as altered buoyancy after a natural fasting period. Although we now know how Antarctic fur seals are able to compensate for such an experimental manipulation, we need to know how the response of Steller sea lions might be different. Therefore, we cannot simply extrapolate to Steller sea lions from the Antarctic fur seal experiments in which foraging costs were increased by inducing additional hydrodynamic drag. Steller sea lions are different from fur seals in many important ways, including body size, oxygen storage capacity and preferred foraging habitat. These differences are sufficient enough to require that we conduct experimental manipulations directly on Steller sea lions.

Conducting this experiment with Steller sea lions from the endangered Western DPS is justified because of the unique biological characteristics of Steller sea lions and the unique foraging habitats of Western stock sea lions compared with Eastern stock sea lions. Extrapolation from the results of other pinniped species would be of limited value for trying to predict the effects of changing body condition or work load on foraging efficiency and maternal investment because Steller sea lions are unique in being a large otariid that dives benthically but does not routinely exceed its estimated physiological dive limits. Because of differences in physical oceanography (especially bathymetric relief and current profiles) and fisheries oceanography (i.e. the available prey) in the areas occupied by Western and Eastern DPS Steller sea lions, it is important to conduct these studies with Western DPS sea lions. The manipulations that we are proposing will last only a very short period of time, so we would expect that even if Steller sea lions cannot compensate for the increased costs of foraging, any negative effects, such as reduced body mass, will be very small and could be recovered from by compensatory feeding without any adverse affects on survival or reproductive success.

We are also proposing to study the foraging ecology of juvenile Steller sea lions because their survival was clearly reduced during the period of greatest population decline during the 1980's (York 1994). In the last decade, a great deal of attention has focused on juvenile Steller sea lion diving and movements, but unfortunately, telemetry studies mostly relied on instrumentation with poor temporal and spatial resolution of diving behavior with no information collected on foraging success. Studies have shown that juveniles are capable of diving to adult depths by the time they are two years old, but their average depths are much less than adult sea lions (Loughlin et al. 2003; Pitcher et al. 2005). Mean dive depth and duration does increase with age, but it is not clear yet how it

is affected by habitat or prey availability (Pitcher et al. 2005; Fadely et al. 2005). Therefore, we are proposing to instrument juvenile Steller sea lions in order to learn how variations in the environment affect behavior and ultimately survival and future reproduction. Young of the year sea lions will be instrumented in order to study the transition from dependence on their mother to complete nutritional independence. The behavior of independent juveniles will also be studied. Gastric lavage and an enema will be performed to determine whether the juvenile is feeding on its own. Blood and blubber samples will be collected for health and body condition assessment. Instruments will be attached (as for adult females above) to monitor the at-sea foraging behavior and energetics. A subset of juveniles will receive buoyancy/drag blocks that are similar to those used for adult females. Buoyancy/drag blocks will be released from the juveniles after a measuring period of no more than four weeks. Archival instruments will be remotely-released after measurement periods of up to one year. Juveniles may be recaptured as many as two times to allow subsequent measurements of body condition and health status.

Another method that may be useful for determining whether young sea lions are still suckling is blubber biopsy collection. The blubber samples would be analyzed to examine fatty acids to obtain information on the diet and weaning status. This work will be done in collaboration with Dr. Lorrie Rea of the Alaska Department of Fish and Game (ADF&G). Dr. Rea's laboratory will conduct the fatty acid analysis. This laboratory has determined that it may be possible to determine weaning status from quantitative fatty acid signature analysis. We will attempt to validate this method by comparing our direct measurements of prey ingestion from instrumented sea lions. If it is not possible to recapture some of the instrumented individuals, we will retrieve their instruments using the mounted remote-release device. However, in order to get blubber biopsies from these individuals, we will need to use blubber biopsy darts that are fired from an air-powered rifle or cross-bow. This technique has been used successfully on Steller sea lion juveniles and adults to collect blubber biopsies for genetics and food habits studies (Hoberecht et al. 2006; Burkanov pers. comm.).

We also plan to use a remotely-controlled vehicle to obtain samples and to make measurements of body condition. The remote-controlled vehicle will be battery powered and very quiet. It will be camouflaged so that it looks like a rock. It will be designed to act like a turtle in that its external shell can be lowered down to the substrate, in effect it will "hunker down" when not moving. It will be controlled by a hidden operator. The remote-vehicle will be used to move about the rookery or haulout to collect samples such as scat, placentas, carcasses or organs and regurgitated milk from pups. It will also be used to remotely measure blubber thickness in adults and juveniles by incorporating an ultrasonic probe. To measure blubber thickness, the remote-controlled vehicle would be directed to move up to a sleeping sea lion, moving close enough for the probe to make a brief (~1 sec) contact with the skin of the sea lion, then move away. Sea lions that receive this remote blubber thickness measurement will also be marked with a dye or bleach that is applied to the fur by the remotely-controlled vehicle.

Remote video monitoring

To have a complete understanding of Steller sea lion life history and factors that may be affecting population recovery, it is important to have long-term knowledge of seasonal population trends at rookeries and haulout sites, reproductive rates of individual females, the extent of maternal care and the extent and nature of pup mortality, including predation. While conducting direct, continuous, long-term observations at rookeries and haulout sites is critical for the assessment and proper management of Steller sea lion populations, collection of such data is difficult because of the remote nature of many locations which makes it impossible for researchers to remain on sites for extended periods and during times of inclement weather.

In 1998, ASLC began a remote video project on Chiswell Island near the entrance to Resurrection Bay to count sea lions and conduct behavioral studies. This work was conducted under a Fish and Wildlife Service permit to ASLC (#01-015) and Office of Protected Resources permit issued to the National Marine Mammal Laboratory (#782-1447, 782-1532). Video cameras and supporting equipment including a microwave transmitter and solar and wind generators were installed on Chiswell Island adjacent to the Steller sea lion rookery. Up to six cameras equipped with pan, tilt and zoom functions are placed at intervals above the rookery for complete viewing of the sea lions using this area. Audio and video signals from those cameras are received and controlled in real-time from ASLC (Maniscalco et al. 2006). Remote video sites have since been installed at haulouts near the Chiswell Island rookery at Seal Rocks (Kenai), Natoa Island and Cape Resurrection Islets to assess behavioral patterns such as foraging cycles, timing of weaning and spontaneous abortions at those sites. Few (3–8) annual visits are currently needed to service and maintain the remote video system. Those visits often occur during times of year when sea lion presence is low or non-existent, making this an ideal project for low-impact, long-term monitoring of a rookery and haulouts in the endangered stock.

Maternal Care

Research associated with these remote video studies builds upon past research and also assesses new aspects of Steller sea lion biology. Studies of maternal care in Steller sea lions have provided indirect evidence that the Western DPS is not currently experiencing food limitation based on observations of lengthy perinatal periods and short foraging trip durations (Maniscalco et al. 2006; Milette and Trites 2003). However, interseasonal and interannual variations exist, (Maniscalco et al. 2006; Trites and Porter 2002) making long-term datasets critical for determining associations with changes in birth rates and other population parameters.

Reproductive Rates

Reproductive output over the lifetime of a species is one of the key parameters in determining population viability and growth potential, and longitudinal studies of individual animals can provide the most detailed information in many cases (Clutton-Brock 1988). Remote video studies at ASLC are beginning to build a long-term dataset (6 years) on the reproductive performance of individual female Steller sea lions and preliminary results suggest that most females attempt to reproduce every year. However, females that do not give birth or lose their pup in one year may be less likely to give birth

the following year compared to females that give birth and suckle throughout the winter (Maniscalco et al. 2005). These data could have serious implications for the recovery potential of the Western DPS and should be scrutinized over a greater proportion of the reproductive lifespan of female Steller sea lions, about 10 or more years (Pitcher and Calkins 1981).

Mortality

Steller sea lion mortality and survival are also being assessed through remote video studies at ASLC (Maniscalco et al. 2005). Most long-term studies of survivability in pinnipeds are extremely valuable (e.g., Pendleton et al. 2006) but rely on marked populations. Yet, little is known about the extent and nature of mortality prior to the time sea lions are marked at 1 to 2 months of age. This may be one of the most critical time periods for survival in Steller sea lions. Studies have found early mortality rates in Steller sea lions to vary from 3-6% (Aumiller and Orth 1979; Lewis 1987; Merrick et al. 1988) to 20% or more (Calkins and Pitcher 1982; Kaplan 2005). Some of those studies suggested that storm waves were the greatest source of pup mortalities; however, weather conditions can vary greatly from year to year. Those studies could only speculate as to most of the causes for additional mortality such as trauma related injuries, stillbirths and abandonment. Our remote video studies provide direct observations for most causes and for the amount of pup mortality at Chiswell Island (Maniscalco et al. 2005). Continued observations along with collection of dead individuals will provide a more complete assessment of the nature and extent of pup mortality and how it may be affecting the recovery of the Western DPS.

Predation by killer whales (*Orcinus orca*) has been one of the major hypotheses for the cause of Steller sea lion population declines in the Western DPS (Springer et al. 2003; Williams et al. 2004). At the Chiswell Island rookery, killer whale presence and predation has been assessed through remote video monitoring, and, in conjunction with other field studies, it has been suggested that predation is not greatly affecting the recovery of Steller sea lions (Maniscalco et al. *In Press*). However, there has been much interannual variability in estimated predation at this rookery which ranged as high as 23% of pups born in a single year (Maniscalco et al. *In Press*). Therefore, because of the great degree of interannual variability, long-term monitoring of killer whale predatory patterns at these Western DPS rookeries is crucial to determining the true effect that these cetaceans are having on the recovery of Steller sea lions.

Reproductive Failure

Reproductive failure in the form of spontaneous abortions may be the result of poor health in female Steller sea lions (Pitcher et al. 1998). We have observed some abortions during the winter and spring months at our remote-video monitored haulouts (ASLC unpublished data). We plan to continue to monitor the occurrence and numbers of these aborted fetuses and collect them when possible to examine for bacterial infections, contaminant loads and endocrinology.

Steller sea lions appear to have very low reproductive success compared to other pinnipeds. It is estimated that the majority of the adult females are successfully impregnated each breeding season, but only approximately two-thirds of these females carry their pups to term (Pitcher et al. 1998). Reproductive failures are characterized by

re-absorption of the fetus early in pregnancy and abortions later in the term. One suspected cause of pre-term reproductive failure is bacteria such as *Leptospira* and *Brucella*, which have been closely associated with reproductive failure in pinnipeds (Smith et al. 1974; Rhyan et al. 2001). While they have been found in other pinniped species (Bricker et al. 2000; Nielsen et al. 2001; Colagross-Shouten et al 2002; Calle et al. 2002), the prevalence of these and other bacteria in the Steller sea lion have not been thoroughly investigated. Opportunistic collection of aborted fetuses will allow us to document and examine this phenomenon, as both *Leptospira* and *Brucella* are commonly found concentrated in aborted fetal and placental tissues in a variety of mammals (Malone et al. 1997; Donahue and Williams 2000; Gidlewski et al. 2000; Rhyan et al. 2001; Guitian et al. 2001).

Another suspected cause of reproductive failure in Steller sea lions is the role played by environmental contaminants in both reproductive disruption as well as fetal exposures. It has been shown that xenobiotics such as DDT, dioxin and polycyclic aromatic hydrocarbons have a deleterious effect on adult reproduction and fetal organ development in a wide variety of species (Wiig et al. 1998; Hoyer 2001; Washington et al. 2001; Hoekstra et al. 2001; Akingbemi and Hardy 2001; Moran et al. 2001; Gotz et al. 2001; Safe et al. 2001). Environmental contaminants, polychlorinated biphenyls (PCBs) in particular, have been found to interfere with the immune response in laboratory animals through the hyperadrenalcortical effects, causing chronic corticoid release and subsequent suppression of B cells (Munck and Crabtree 1981; Fuller and Hobson 1986). A feeding study of harbor seals that was conducted over a 2½ year period showed there was a positive correlation between high levels of organochlorines and suppression of natural killer cells and specific T-cell activity (de Swart et al. 1993, 1994, 1996). Immunosuppressive effects of organochlorines are also indicated in several different studies of striped dolphins affected by the morbillivirus epizootic of 1990 in the Mediterranean Sea (Aguilar and Borrell 1994; Troisi et al. 2000, 2001; VanLouveren et al. 2000). Moreover, because it has been found that organisms will exhibit species differences (often to the level of organ specific) in enzyme systems to metabolize different OC compounds (Passivirta 1991; Boon et al. 1994; Reijnders 1994; Rice and O'Keefe 1995), generalizations regarding the effects of organochlorines can not be made between species. In other words, a chemical found to be harmful in one species may be readily metabolized and cleared from the system of another. Opportunistically collected placentas will also be examined for basic physiological structure and function, enzymology, contaminant load, immunoglobins and endocrinology. Aborted fetuses and deceased newborns (0-30 days old) will similarly be examined for enzymology, contaminant load and endocrinology.

Branding Studies

Having a reliably and permanently marked population of animals of known age and origin can provide a wealth of data on population parameters such as age-specific reproductive rates, survival and migration/movements. Hot-branding has proven to be the best method for permanently marking Steller sea lions (Merrick et al. 1996) and has already provided many researchers with the ability to assess important aspects of sea lion behavior and survival (e.g. Raum-Suryan et al. 2002; Pendleton et al. 2006). Since the installation of the remote video system, the sea lions at Chiswell Island are one of the

most well studied and understood populations in terms of maternal care (Maniscalco et al. 2006) and reproductive performance (Maniscalco et al. 2005). Having animals at this rookery of known age and origin would give a more complete picture of those key aspects of Steller sea lion biology than is possible anywhere else. Hot-branding sea lions as pups is the only way to obtain a reliably marked adult population at Chiswell Island in future years.

Hot-branding as a form of permanent marking is not thought to adversely affect survival in pinnipeds (Merrick et al. 1996; McMahon et al. 2005); although, it has become a very contentious topic with some animal rights groups. Another benefit of branding sea lions at Chiswell Island is provided by remote video observations that will be able to easily assess the behavior, apparent health and survival of all animals for up to several months after researchers have departed the rookery. Such detailed studies of the effects of invasive research and research associated disturbance are not possible in other locations and are invaluable for providing essential data for studies such as Environmental Impact Statements.

Blood and other material collections

Basic hematology, clinical chemistry and stress response panels from Steller sea lion pups will allow us to identify possible infections and/or abnormalities that can affect sea lion health. The immune system protects the body from potential infections and is critical for health and survival of an individual. There is a well-established bi-directional communication between the immune and endocrine systems (Haddad et al. 2002; Weber 2003). These finely tuned interactions are required for the survival and health of an individual and are regulated by circulating hormones and cytokines, which are in turn influenced by body condition and stress. The potential impact of stress on an animal's ability to survive may be greatest during times of high energy demands such as pregnancy, lactation and periods of development and rapid growth. We will quantify hormones previously shown to indicate stress and/or influence immune function including cortisol, aldosterone, leptin and thyroid hormones.

At birth, the mammalian immune system is not fully developed, requiring the temporary reliance on maternal antibodies received via colostrum until their immune system matures. A delay in the development and/or suppression of the immune system has been shown to lead to an impaired cell-mediated immune response, lasting months to years (Chandra 2002). Suppressed immune function has been associated with reduced number of T lymphocytes and reduction in their response to mitogens (Chandra 1991). T lymphocytes are an important component of the cell-mediated immune response whose function is driven by the presence of circulating antibodies. A compromised cell-mediated immune function would lead to an increase in susceptibility to disease and ultimately mortality and may be the result of low birth weight and/or inadequate transfer of immune protection from the mother. Lymphocytes are an important adaptive cellular component of the immune system. The capacity of lymphocytes to proliferate in response to an antigen is central to the success of the adaptive immune system. Cell-mediated immune function can be assessed *in vitro* with the lymphocyte proliferation assay (LPA). LPA is a well-established assay for measuring the cell-mediated immune function and is validated by the National Toxicology Program (Luster et al. 1988). LPA

is increasingly used to assess immuno-competence in pinnipeds (de Swart et al. 1993; Levin et al. 2005; Mori et al. 2006).

While it has been determined that two genetically distinct stocks of Steller sea lions exist in Alaska (Bickham et al. 1996, 1998), skin samples will be analyzed in conjunction with the National Marine Mammal Laboratory (NMML) to expand their existing genetic databases. This information will allow us to further determine the amount of genetic diversity between and within stocks.

Carcass analysis can provide a wealth of information for a given species. Age at mortality and potential causes for mortality can be inferred. In addition, carcasses can provide large depot sources of tissues required for laboratory research, reducing the amount of invasive disturbance to the animate population. Steller sea lion carcasses periodically become available through field research teams from NMFS, ADF&G, The North Pacific Universities Marine Mammal Research Consortium and the Alaska Sea Otter and Steller Sea Lion Commission.

Monitoring the diet of Steller sea lions through the analysis of hard parts recovered from scats continues to be an important aspect of examining the nutritional stress hypothesis. A major component of the nutritional stress hypothesis is the question of nutritional quality vs. quantity of prey. In the areas of decline, the diet of sea lions, primary low-energy gadids and Atka mackerel, has not significantly changed since the 1980's (NMFS 2001; Sinclair and Zeppelin 2002), suggesting that these animals may still be subjected to nutritional stress. It has also been shown that the diet diversity of sea lions in Alaskan waters was significantly correlated with the rate of population change (Merrick et al. 1997). Pinniped populations subjected to nutritional stress exhibit an increased mortality level of pups when their mothers are not able to provide sufficient energy after first meeting their own caloric needs (Trillmich and Dellinger 1991; Roux 1997). Additionally, juveniles have higher relative energetic demands (Winship et al. 2002) and may also suffer increased mortality rates due to foraging inexperience. Therefore, determining the diet of adult female and juvenile Steller sea lions is a major step in testing the nutritional stress hypothesis as a cause of the Steller sea lion decline. Combining hard-part analysis with radioimmunoassay (RIA) and high pressure liquid chromatography fecal glucocorticoid (HPLC-FG) analysis allows for a greater depth of understanding in prey selection for this species.

An additional study based on scat analysis involves a survey of corticosteroid concentrations in scat collected from rookeries previously shown to have high concentrations relative to those rookeries in close proximity. A pilot study that examined scats collected across the range of the Western stock indicated that animals on rookeries close to Unalaska (Ugamak and Clubbing Rocks) had high concentrations of the stress hormone corticosterone when compared to areas close to these sites (Atkins, Whaleback, Cape Morgan) (Mashburn and Atkinson 2004). Because these samples represent single point data, it is not possible to conclude that sites with high corticosterone values represent "stressed" areas. In order to determine whether heightened adrenal activity in the areas closest to Unalaska is both chronic and not due to natural stressors such as weather and predator presence, the proposed study will collect scat from these areas, as well as the surrounding "low" areas longitudinally.

Task 2

Steller sea lion populations have been in flux for several decades, mostly dramatically with a decline in the Western DPS. Through a concerted effort of many agencies, the potential causes for the decline have been investigated, including disease, predation, environmental change and competition with fisheries. Focus age groups have shifted from neonates to juveniles to adults and juveniles. While it is unlikely that a definitive cause of the decline will be uncovered, it is clear that we need a deeper understanding of the basic physiology and plasticity of response to perturbation in this species. For instance, can juvenile animals respond in a sustainable fashion to reduced prey availability? Does reduced foraging success have immediate effects on survival, or does it instead influence reproduction in the adult years? In addition, advancements in technology have the potential to provide long-term monitoring and non-invasive condition assessment methodologies that previously were not feasible.

The Transient Juvenile Steller Sea Lion Project was implemented in 2001, and it has since supported over a dozen projects from internal and external researchers focused on the nutritional and health status of juvenile Steller sea lions from the northern Gulf of Alaska (e.g., Calkins et al. 2005; Mellish et al. 2006; Mellish & Horning 2005; Mellish et al. in review; Schrader et al. 2005; Stephens et al. 2005; Thomton & Mellish in press; Waite et al. in review). The program and facility are outlined in detail in Mellish et al. (2006). The unique nature of the specialized quarantine facility at the Alaska SeaLife Center allows for the safe and effective study of multiple wild-caught individuals in a captive setting for periods of up to three months. The temporarily captive nature allows for detailed studies of health, physiology and nutrition, which provides essential and previously unavailable baseline information for interpretation of field collected data. It is also the ideal situation for testing new monitoring devices as well as validation of non-invasive techniques to assess condition. Field research typically allows for a single assessment of an individual, which cannot provide the baseline data needed for many physiological studies (e.g., stable isotopes, fatty acids). Currently, the Transient Project supports studies that fall within three categories: a) general health and nutrition, b) validation of new technology and c) experimental manipulation. The categories are not mutually exclusive, and indeed, attempts to accomplish multiple objectives with minimal procedures are made whenever possible.

Health and Nutrition

As noted in the Steller Sea Lion Recovery Plan (National Marine Fisheries Service 2006), assessment of the general health status of the population is an ongoing priority. The Transient Project approaches this need from a variety of angles. First, a control set of individuals (up to 10 per event) are sampled for complete blood count, serum chemistry, viral serology, body condition, nutritional status and parasite load to determine the typical condition of the juvenile population being sampled at the time. This serves as control comparisons for the transient individuals as well as an ongoing census of the region. Particularly in the late winter/early spring months, it is difficult to discern pups of approximately 8-10 mo from juveniles > 1 year prior to anesthesia and examination of tooth eruption patterns (King et al. 2004), such that pups may be sampled. However, pups are released in the field and are not included in the transient cohort. Temporary

captivity is limited to animals estimated to be greater than one year of age. In addition to routine CBC and chemistries, both innate and acquired immune function will be accessed in vitro with lymphocyte proliferation, phagocytosis and respiratory burst assays.

While we are not selecting specifically for healthy individuals, we will exclude animals with any major outstanding health issues that might lead to increased stress during temporary captivity (e.g., broken bones, major infection) or conditions that might result in transmission to other individuals (e.g., heavy parasite load). The health screening protocol used at entry and exit is outlined in **Appendix A**. During temporary captivity, animals are monitored and sampled up to weekly for health and research requirements. Whenever possible, samples and handling events are shared among priorities and investigators to greatly reduce the overall amount of handling required. Prior to release, animals are outfitted with scientific instruments and permanently marked via hot-brand to increase our ability to re-sight and monitor post-release behavior. Satellite tags are typically utilized to provide data on location and dive depth/duration. However, tags are only useful for the lesser of the battery life or interval to molting. Hot-branding has been a controversial procedure but is considered by many scientists in the field to be the most effective method for long-term monitoring and the best tool for population monitoring (National Marine Fisheries Service 2002). A recent study has shown that despite the visually unappealing healing process, juvenile sea lions are fully capable of rapid recovery from the procedure (Mellish et al. in review).

Technology

Life History Transmitters (LHX)

We will implant dual, satellite-linked Life History Transmitters (LHX tags) into up to 90 juvenile Steller sea lions over five years, while in temporary captivity at the ASLC transient juvenile facility, prior to release of the animals. LHX tags were specifically developed for long-term monitoring of large marine homeotherms (Horning & Hill, 2005). The devices are implanted into the peritoneal cavity using standard sterile surgical procedures while the animals are under gas anesthesia. LHX tags monitor state and behavior of implanted animals throughout their life. After extrusion from the decomposing or partially consumed carcass, the tags float to the surface or fall out ashore and uplink previously stored data via the ARGOS system aboard National Oceanic and Atmospheric Administration (NOAA) satellites. LHX tags provide data throughout the life of the animal on weekly cumulative foraging effort and time and date of death, as well as information on temperature gradient from two days prior to two days following death. From this temperature data, inferences can be made on the occurrence of traumatic death vs. non-traumatic death. Dual LHX tag implants will be used to calculate tag failure rates. In conjunction with carcass testing for the purpose of assessing the effects of non-independence of dual tags on failure rate determinations, this will provide correction factors required to accurately estimate survival rates.

Data from LHX tags will be used to assess survival rates, to relate individual survival to health status at time of release, multi-year and seasonal foraging effort and separately assessed environmental data. LHX tags provide the equivalent of individually, spatially (unlimited geographic coverage) and temporally unlimited (1 day resolution) resight effort, compared to classic mark-resight approaches based on semi-permanent (flipper tags) or permanent marking (hot branding), combined with limited visual or

VHF-telemetry resight efforts. As a result of the unlimited resight effort, multiple hypotheses on the relationship between specific parameters and survival can be tested with sample sizes several orders of magnitude smaller than using mark-resight approaches with spatially, temporally and individually constrained resight effort. In addition, LHX tags provide data on the fate of individual animals, allowing direct comparisons between animals that survive for differing periods of time. This will allow testing specific hypotheses with much smaller sample sizes than the frequently used regional comparison approach, based on the different likelihood of sampling long-term vs. short-term survivors in distinct population segments (DPSs) with divergent population trends. In addition, information on the nature of mortality events of individual animals (traumatic vs. non-traumatic) will contribute to the assessment of the relative contribution of top-down vs. bottom-up effects.

Deployment of dual LHX implants on transient juvenile Steller sea lions prior to release constitutes the initial phase of the Steller sea lion LHX project. For the LHX project, sample size estimation suggests a minimum sample size of 72 animals to allow testing of significantly reduced juvenile survival as a prime contributor to population trends in the Western DPS. These estimates were derived from conventional, parametric power tests based on an analysis of variance under a number of assumptions that may not necessarily be equally met by conventional mark-resight or LHX based survival rate estimates. This makes a direct comparison of the efficacy of different techniques difficult to conduct. New statistical techniques to address this limitation are being developed (Horning & York 2006). We project implanting LHX tags in up to 100 juvenile Steller sea lions. The ASLC Transient Juvenile Program allows for the deployment of LHX tags under highly controlled conditions, and we propose to implant dual LHX tags as a standard exit procedure for all transient juveniles. This will allow the testing of a number of additional hypotheses, including the long-term effects of temporary captivity on individual survival and of hot-branding on long-term survival (if only a subset of transients will be branded).

To date, four rehabilitated California sea lions (at The Marine Mammal Center, Sausalito, CA) and six juvenile Steller sea lions (at ASLC) have received LHX implants and have been released after observation periods in captivity ranging from four to ten weeks. All ten animals tolerated the procedures well and showed no evidence of infections or other health complications or of altered behavior (Petrauskas, Mellish, Horning, unpublished data; Mellish, Horning, Tuomi and Haulena, unpublished data). All animals were monitored post-release using externally attached satellite telemetry transmitters. Post-release monitoring periods ranged from 10 days (California sea lion) to four months, likely as a function of molt-related loss of external tags. Two of the Steller sea lions were re-sighted using remote video monitoring equipment, based on brands, up to six months after release (seven months after receiving implants). To date, no data has been returned from any of the LHX tags, indicating that all ten animals are likely still alive (within the constraints of the continuing failure rate assessments). Thus, intraperitoneal implantation of dual LHX tags appears to be a viable and well tolerated procedure, as demonstrated on two species of sea lions.

Digital imaging technology

Thermal regulation is a critical process for marine mammals, as they must be able to function in two metabolic media, water and air. In order to maintain body heat in cold waters, most marine mammals must be well insulated. With the exception of sea otters, most marine mammals do this through an insulative layer of blubber just under the skin. The way in which animals store blubber can have implications for how they manage heat retention in cold environments and heat loss in warm environments. Development of non-invasive technologies allows us to remotely estimate mass (e.g., Waite et al. in review), investigate patterns of blubber distribution (imaging ultrasound) and heat flux (thermal imaging) in pinnipeds. Body condition is often used as an overall measure of animal health for which blubber depth can be used as a non-invasive index. However, baseline information on variations in energetic status and blubber depth in these species are necessary prior to full utilization and interpretation of this measure in the field.

Portable ultrasound technology has advanced significantly over the past decade, with the current production of instruments that are logistically capable of being utilized in field situations. Validation studies of the use of ultrasound have shown accuracy to 99% of the actual for measurement of blubber depth (Mellish et al. 2004). In a previous study, we demonstrated that there are both seasonal and species differences in the way that individuals store and utilize blubber (Mellish, Horning and York, in press). We now propose to take this study to the next step, through the pair-matched addition of thermal imaging data. While ultrasonic measurements of blubber depth can help us infer body condition and suggest patterns of insulation, the thermal imaging tool can allow measurement of heat flux. This will facilitate a detailed examination of how blubber depth does or does not correspond to actual heat loss in individuals over time.

Video Data Recorder (VDAP)

The Recovery Plan for Steller sea lions (National Marine Fisheries Service 1992) and the National Research Council (2003) have identified the need to identify habitat requirements and areas of biological significance for Steller sea lions and to investigate feeding ecology. Specific points include: map, describe, and evaluate feeding areas; determine seasonal use patterns; refine understanding of Critical Habitat use; identify feeding areas and investigate diving behavior and feeding cycles. 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. We propose to study the hunting behavior and three-dimensional movements of juvenile sea lions via a small video system/data. To characterize Steller sea lion habitat-associations, we will combine satellite tracking and dive data with bathymetry and TOPEX/POSEIDON and ERS satellite remote sensing of hydrographic features. This study will provide fundamental information on the foraging ecology of Steller sea lions and enable us to compare general foraging strategies, searching mechanics, modes of locomotion and foraging efficiency at different rookeries and at different times of the year. The results will address questions of prey preference, predator/prey relationships and ecological attributes of foraging habitat. In addition, we will assess the abundance, distribution and composition of prey at spatial and temporal scales pertinent to foraging juveniles. Availability and quality of food resources is a

likely mechanism for influencing the survival of juveniles and young of the year, especially during the winter. Up to five transient juveniles per year will be outfitted with video/data acquisition platforms (VDAP) recorders within one-week of release, with instruments to be recovered via remote release. In addition, up to five transients may be temporarily fitted with VDAP recorders for up to two weeks during the temporary captivity period in order to study best attachment and mounting technique.

Experimental studies

In addition to the validation of new technology, the Transient Project provides the best scenario for controlled manipulations for physiological studies that require repeated handling, dry holding, or temporary food restriction. Animals can be monitored up to 24 hours a day in a controlled, quarantine setting.

Restricted intake/fasting

Previous fasting studies at the South Beach facility have shown that there may be disparate responses to energetic deficit depending on body condition at the beginning of the fast (Mellish and Horning, in review). Building on these studies, we will closely monitor changes in fasting metabolites (e.g., free fatty acids, blood urea nitrogen, ketone bodies) as well changes in basal metabolic rate and thermoregulation via heat flux signatures. Juveniles of poor body condition or small body mass may be less equipped to deal with periods of poor prey accessibility or foraging success. Information gathered through non-invasive monitoring of body condition, basal metabolic rate and heat loss will allow us to better understand the physiological mechanisms that may help individuals cope with a negative energy balance and what the limitations are of those responses. Variations in initial body mass, condition, season and sex may play important roles in the response to an energetic challenge.

Stable isotopes as dietary markers

Nutritional and diet information is critical for the accurate interpretation of prey selection in the wild. New applications of fatty acid blubber analysis and stable isotope evaluation (e.g., whiskers) may shed light on the short-term and long-term trophic selections of Steller sea lions. While validation studies for the signature period to vary exists for some species typically measured via fatty acid analysis (e.g., Kirsch et al. 1998), the temporal scale for changes in stable isotope levels in pinniped whiskers is less understood (Hirons et al. 2001). Careful monitoring and selection of differing trophic level diets for varied time periods during the temporary captivity period of transient juveniles will allow for a finer-scale analysis and interpretation of the field-collected samples.

3. Hypothesis/Objectives and Justification

The justification for each of our research objectives has been provided in the above section along with the background of the problem, expected significance and contribution to recovery plans. Here, we state the specific objectives and hypotheses.

Task 1

The overall objective of our research on free-ranging Steller sea lions is to further the understanding of Steller sea lion biology and to attempt to determine the factors that may be limiting the recovery of the Western DPS. Specifically, our goal is to conduct detailed investigations into behavioral and physiological ecology in order to assess how environmental variation affects the behavior, condition, survival and reproductive success of individual sea lions.

There are two main objectives of our research on the foraging ecology of Steller sea lions:

1. Determine the mechanisms that link foraging success to reproductive success in adult female Steller sea lions.
2. Examine the transition from maternal dependence to nutritional independence in young Steller sea lions.

We will test the hypothesis that Steller sea lion population recovery is currently limited by nutritional stress by making observations of the foraging behavior and energetics of juvenile and adult female Steller sea lions while they are at sea and on shore. We will more directly measure the consequences of different behaviors by directly measuring maternal investment through milk intake and pup growth and survival and the ultimate reproductive success of the offspring.

Because our objective is to determine how Steller sea lions might be affected by changes in the availability or distribution of their prey, we must study live Steller sea lions in their natural habitats. We will use computer modeling and laboratory simulation of the response variables whenever possible, but we have already determined through modeling that we do not have enough basic information on many of the parameters that are needed to accurately model the energetics and behavior of sea lions. We cannot rely only on research on surrogate species because our research is designed to provide information to aid the recovery of this particular species. There are many characteristics of Steller sea lions, such as size and behavior, that differ significantly from the values for possible alternative species. We have conducted similar studies in the past and these have provided us with an estimate of the amount of variation that we can expect in many of the parameters that we plan to measure. Power analysis has demonstrated that for many of the variables we will need even larger sample sizes than the ones we have proposed in order to detect slight differences in response to the anticipated variation in prey availability within one year. However, because the capture and instrumentation of Steller sea lions is logistically difficult and very expensive, we hope that even with a limited sample size that we will gain extremely valuable data and insights.

The main objectives of remote video monitoring project and associated studies are to provide a complete understanding of reproductive performance, maternal care and mortality in Steller sea lions. These studies will provide assessment of potential problems that may inhibit the recovery of the Western DPS.

Specific hypotheses or questions to be tested:

1. What are the lifetime female reproductive output potentials?
2. What factors affect the reproductive potential of females?
3. What is the nature and extent of mortality in pups, including predation?
4. How does diet relate to and affect maternal care?

5. How does branding affect the behavior and survival of pups and the behavior of mothers toward their pups?
6. What is the variation between rookeries in stress hormones found in scat?

Task 2

a. The overall objective of the transient Steller sea lion research program is to aid in the investigation of the decline of the western stock and its failure to recover and to assist recovery efforts through the accumulation of essential information.
Specific objectives include, but are not limited to:

1. Ongoing collection of baseline health parameters of pups and juveniles
2. Temporary captivity for research purposes for up to 30 animals/year
3. Continue post-release monitoring via visual resight (e.g., hot-brand), scientific tag attachment (e.g., satellite tag, video data recorder), and LHX implantation
4. Validate the use of non-invasive tools (e.g., thermal imaging, ultrasound, 3D imaging) for the determination of health and condition indices
5. Perform calibration studies for nutritional baseline analyses (e.g., stable isotopes, fasting metabolites)
6. Examine physiological response to restricted intake/fasting via metabolic chamber, body condition assessment (ultrasound, d2o) and heat flux
7. Study foraging behavior and habitat selection through scientific instrument attachment

Tasks 1 and 2

b. All projects outlined in this permit application are focused on the collection of information crucial for the ongoing Steller sea lion recovery effort. Therefore, the most accurate data will only be available through directed research on this species. The tasks outlined are in response to recommendations from several panels, including the National Marine Fisheries Steller sea lion Recovery Plan (1992, 2006), the Steller sea lion Research Peer Review (Didier 1997a, b), Steller sea lion implant workshop (Horning et al. 1999), the Steller sea lion Physiology Research Workshop Review (Williams et al. 1999) and the 'Is It Food II' Workshop (DeMaster and Atkinson 2002).

C. Methods

1. Duration of Project and Location of Taking

Task 1

The capture, biological sampling and attachment of scientific instruments to study the behavioral and physiological ecology of foraging will take place from July 1, 2007 to June 30, 2012. As indicated in the accompanying Take Table (Table 1), Steller sea lions will be captured from the Western DPS. The choice of which rookeries or haulouts that we capture from will be made approximately 3-6 months before the field work commences and will be based on careful consideration of the following factors: most recent population trends for that site or its region; most recent data on the prey base for the area around that site; whether that site has been disturbed or is expected to be disturbed in the near future by other researchers; and whether collaborative studies can be arranged to reduce cumulative effects of research or to capitalize on concurrent complementary Steller sea lion research or fish stock assessments at or near a site. The

planning of such research will be done in consultation with all of the Alaska SeaLife Center investigators and the other principal investigators that hold the major Steller sea lion field research permits. In the past, the major permits were held by the Alaska Department of Fish and Game, National Marine Mammal Laboratory (NMFS) and Alaska SeaLife Center. The three Principal Investigators holding these permits, Dr. Lorrie Rea, Dr. Tom Gelatt and Mr. Don Calkins, and many of their co-investigators are in regular contact with each other and with the various co-investigators on all permits. This close contact has led to sharing logistics and personnel on a regular basis. In addition, usually one or two of the three coordinate regularly with the other Principal Investigators holding or applying for permits to conduct research on Steller sea lions. In fact, Dr. Gelatt, the National Marine Fisheries Service Permit principal investigator, regularly (at least annually) hosts coordination meetings with appropriate permit holders to help enhance synergistic effects and reduce unnecessary overlap and avoid duplicative research.

Remote video studies will take place on a year-round basis from July 1, 2007 to June 30, 2012 in Resurrection Bay and Kenai Fjords. Dedicated brand-resight cruises and scat and carcass collection will take place opportunistically during that same time-frame in the greater Kenai Fjords/Prince William Sound region. Pup branding and associated health assessments will take place on a single day at the selected rookeries in Table 1, approximately between July 1 and July 5, 2007 and approximately between June 28 and July 5 in each subsequent year from 2008 to 2011.

Task 2

The project will take place from July 1, 2007 to June 30, 2012. Field seasons will occur on a variable schedule throughout the year. Locations of taking will include the northern Gulf of Alaska and the Aleutian Islands. Primary field sites will include Resurrection Bay and Prince William Sound. Only juvenile haulouts and non-active rookeries will be targeted. In most cases, animals will be captured underwater in the vicinity of the haulout location and landings will only be made when necessary during the capture activity (e.g., noosed animal hauls out) or to recover scientific instruments.

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

Task 1

Table 1 outlines all proposed activities for Task 1 (foraging ecology, reproductive performance, remote video monitoring and associated studies) including capture, restraint, morphometrics, digital imaging, blood and tissue sampling, scientific instrument attachment, hot branding, flipper tagging, incidental harassment and carcass/organ collection. For all procedures listed, activities will only be performed by/under the direct supervision of qualified and experienced personnel. We consider that personnel who have received the appropriate training (i.e. they have received instructions from an experienced person and had previously observed a minimum of five events) and have correctly performed the procedure under the supervision of a qualified researcher or veterinarian to have "sufficient experience." An emergency kit with equipment and supplies for responding to complications will be readily available. Whenever possible,

procedures including, but not limited to, blood and tissue collection and hot-branding will occur while the animal is under general anesthesia in order to reduce potential stress to the animal, as well as increase the safety of the individual and the handlers.

Capture, restraint and anesthesia - We will capture up to 240 Steller sea lions aged 5 days to 2 months from the Western DPS annually. We will capture up to 40 Steller sea lions aged 2 months to 1 year from the Western DPS annually. We will capture up to 40 Steller sea lions aged 1 year to 4 years from the Western DPS annually. We will capture up to 40 adult female Steller sea lions from the Western DPS during June and July and up to 60 adult female Steller sea lions from the Western DPS between August and May annually. We will capture Steller sea lions on land using nets or lasso, underwater via lasso, or by floating trap. The decision as to which capture method to use will be based on the behavior of the sea lions, their location, the topography of the substrate when they are on land or the sea state when they are in the water. Whether underwater or on land, the lasso will be held on the end of a pole and placed around a sea lion, slightly anterior to the fore flippers. The lasso will be tightened as the sea lion moves away from the capturer, and the rope is then retrieved by personnel in a waiting skiff. Animals are wrapped in a restraining net and pulled into the skiff directly into a capture cage.

We will also capture Steller sea lion juveniles (> 2 months old) by encircling them with a modified purse seine. Small groups (< 50) of Steller sea lions will be encircled by a purse seine deployed by a seine skiff. The purse seine netting will be rugged material with a mesh size small enough to prevent a flipper or head from passing through the mesh. Upon closure of the seine, sea lions will be removed from the net by lowering a brailer into the net and "dipping" for a single sea lion. Once caught in the brailer, the brailer will be hoisted hydraulically and then set down into a capture cage on the deck of the boat. Once in the cage, sea lions will be restrained by squeezing; then, gas anesthesia will be administered using the same methods that we currently use after sea lions are netted out of the water as part of the noose capture method. The method of netting and lifting pinnipeds out of the water with a brailer has been used successfully to capture Australian fur seals for research purposes (Dr. Simon Goldsworthy, South Australian Research and Development Institute, personal communication). Herring fisherman have also described the unintentional capture of Steller sea lions in purse seines and report that sea lions are not harmed when hoisted onto the deck, as long as the net is monitored to avoid entanglement and drowning. A similar in-water net capture method is used routinely to capture other pinnipeds, such as grey seals (Goulet et al. 2001) and harbor seals (Small et al. 2005). Our research group has experience in net captures of harbor seals, and we will employ similar techniques to ensure that this is a safe method for sea lions. The circumference of the net will be monitored by crew in multiple small boats so that any sea lions that do become entangled can be freed by cutting away the net with fisherman's net knives attached to long poles. Sea lions that become too entangled and might be left with some attached netting after cutting the net will instead be lifted by the net and hauled into the small boat, just as we do with noosed sea lions. At any time, sea lions can be released from the purse seine by pulling the two ends apart.

In addition, we will utilize the floating platform capture method, which typically employs a 12-ft. wide buoy with a 12-ft. by 12-ft. platform for a haul-out surface. There are 6-ft. high steel cage walls around the perimeter of the platform, with a wide trap door

on one side. Sea lions haul out and return to the water freely through the trap door. To capture sea lions, the trap door is dropped when sea lions are hauled out inside. Captured sea lions are transferred into a holding cage on 30-foot barge that docks with the floating trap cage and then moved one at a time from the holding cage into a stainless steel squeeze cage. This system is analogous to handling runs and squeeze cages used for livestock on farms and ranches. The squeeze cage restricts the movement of the sea lions without harming them or exposing handlers to unnecessary risk. A sea lion is "captured" by this method only if it is moved into the adjoining squeeze cage and handled for sampling and instrument attachment. At the end of the capture operations, the main trap door will be opened and sea lions that are not sampled can exit the floating platform. Sea lions that are released from the cage without any sampling or other restraint are considered to be incidentally disturbed (see Table 1).

Once a sea lion has been placed into a single animal capture cage or a squeeze cage, it will be anesthetized (see below) in order to weigh and measure it, attach recording instruments (see below), apply tags, draw blood and collect other biological samples. When all procedures are completed and the squeeze cage is opened, the animal is released, and the next animal is allowed in.

Chemical Immobilization of Adult Females – Adult females will be immobilized on rookeries by darting with Telazol® (Tiletamine HCL and Zolazepam HCL, Fort Dodge Laboratories, Fort Dodge, IA) and atropine (Heath et al. 1996). The dart will be delivered by a blow-pipe if the sea lion is located within 3 meters of the darter, or by dart rifle (Heath et al. 1996). After initial observation, a sea lion will be chosen based on its appropriateness for darting. Important factors include presentation of a clear target with limited possibility for obstruction of shot, level of alertness and proximity to the ocean shoreline. An estimate will be made of the target's mass, and the dart will be loaded to achieve a Telazol dosage of approximately 2.0 mg/kg and an atropine dosage of 0.02 mg/kg. After firing the dart, the sea lion will be closely observed until it shows clear signs of immobilization, at which time it will be quickly intubated and anesthetized with isoflurane using a modified large animal anesthesia machine (see below).

If a sea lion responds to darting or the presence of the darter by fleeing towards the water, we may attempt to prevent it from entering the water by capturing it with a thrown or remotely projected capture net. This will only be done if the substrate is a relatively smooth rock or sand surface. The net will be launched from a commercial net capture gun as long as it is far enough away from other sea lions so that only the targeted animal will be entangled in the net. A tether will be attached to the net so that upon contact with the sea lion the net can be pursed closed by applying tension to the tether line. The tether line will be attached to a buoy so that if the sea lion does make it into the water the line can be quickly retrieved and the netted sea lion can be pulled aboard our capture skiff.

For females that are hauled out too close to the water to be deemed safe to attempt chemical immobilization, we will place a noose over the sea lion's head as is done in the underwater lassoing method. This is essentially a combination of the hoop net and underwater lasso methods. As in using a hoop net, we would approach an animal either from the water's edge or from a concealed location on land, but instead of having a net attached to the end of the pole, we would have the exact same type of lasso/noose that we use for the dive capture technique. The noose is attached to a long line with a buoy on

the end of it just as is done with the underwater noosing. Once the noose is placed over a sea lion's head, the buoy and rope are tossed into the water. We fully expect the sea lion to immediately jump into the water because every sea lion that we have observed to become spooked near the waters edge does exactly that. Once the sea lion is in the water with the noose around it, the capture technique will proceed like the traditional dive capture noosing technique. Because the largest animal that has been captured with this method is about 280 kg, and females can sometimes weigh more than this, we will modify the method by which sea lions are brought aboard the boat. The current method is to pull the sea lion into a blanket-type net and then to roll it into the boat. Instead, we have designed a cone shaped net that can be placed over the head of the sea lion when it is brought close to the edge of the boat. The net is tubular and will fall down to the rear flippers where it can be pursed. Once pursed at the bottom, it can be tightened at appropriate points along its length to help restrain the foreflippers of the sea lion. Then, the net can be lifted with the aid of a small davit at its reinforced lifting points. We feel that this slight modification of technique will result in fewer disturbances to other sea lions and is safe for the targeted sea lion and for us. Therefore, this method should result in a lower level of impact to the sea lions than if we always used the other techniques that we are permitted to use.

Pup Capture/restraint/anesthesia - We will capture Steller sea lion pups on the rookery or by boat when they in the water near the rookery. Up to five personnel will approach the rookery by land and water from different directions to flush adults into the water and corral pups together in a crèche. We will attempt to corral pups so that they are no more than 100 pups in a single tight group. If pups enter the water and stray from the rookery, they will be collected from an aluminum skiff using a hoop net with a long handle and returned to the temporary crèche on the rookery or corralled back to the rookery if capture is not possible. Pups in the crèche will be taken individually to sampling and branding stations using hoop nets. All hoop nets will be made of polyester or nylon webbing with a mesh size of about 1" and a ½" diameter aluminum frame. The crèche will be continually monitored to ensure pups do not stray and are not stacked up on each other to prevent accidental injury.

When pups are transferred to the branding/sampling station, they will be physically restrained in sternal recumbancy on a sampling board while a veterinarian or other qualified personnel anesthetizes the animals using isoflurane gas anesthesia with a modified cone held tightly over the face to create an air seal. Pups will be physically restrained by net or hand for not more than 20 minutes and by anesthesia for not more than 20 minutes. All personnel involved in the restraint, anesthetic and research procedures will discuss personnel placement, assignments and duties prior to each event. The veterinarian or veterinary technician in charge of anesthesia will remain stationed at the head of the animal during the entire procedure to monitor respiration, depth of anesthesia and vital signs until the pup has recovered a swallow reflex.

Gas anesthesia (all age and sex classes) - The anesthesiologist will determine when the necessary anesthetic plane has been achieved and verbally notify the research team when sampling, branding or other procedures should begin. Research and support staff will communicate with the anesthesiologist regarding status of manipulations at all times.

Care will be taken to avoid injury around the eyes with the cone. Additional restraint may be achieved by using nylon straps over the body of the sea lion. Animals that cannot be adequately restrained for mask induction will be pre-medicated with intramuscular injections of atropine (0.54 mg/ml) dosed at 0.03 ml/kg and followed at least 10 minutes later by Telazol (tiletamine HCl and zolazepam HCl, Ft. Dodge Lab) at a dose of 0.5-2.0 mg/kg. If only mild sedation is sufficient to allow restraint then Midazolam will be used as an alternate drug for sedation at a dose of 0.2 mg/kg IM. It is possible to reverse some effects of midazolam with an injection of flumazenil (1 ml /10-15 mg midazolam IV or IM). The sedation/preanaesthetic drugs will be administered intramuscularly by pole syringe using the standard 14 gauge x 1.57 inch needle (Daninject) and the appropriate volume syringe. Injection sites will be cleaned, if possible, immediately before placement of the needle by rinsing or swabbing with 70% isopropyl alcohol or a dilute solution of povidone iodine in sterile saline. After administration of injectable sedation/preanesthestic, the anesthetist and sampling coordinator will remain with the animal to monitor induction and observe respiration and behavior. Other personnel should remain away from the area and noise or other disturbance minimized until the animal is fully sedated (about 10 to 20 minutes).

Isoflurane gas will be administered for induction at 5% in medical oxygen with a flow rate of 5 to 10 liters per minute from a properly cleaned and calibrated vaporizer in a closed circuit gas anesthesia machine via the cone mask. Depth of anesthesia will be judged by the anesthetist based on respiratory rate and volume, response to stimuli, palprebral reflex, capillary refill and jaw and muscle tone and maintained using 1% to 3% isoflurane in 5-10 liters of oxygen per minute flow rate as needed. Intubation with an appropriate sized (10-16 mm) cuffed endotracheal tube should be utilized for continued administration of isoflurane gas and oxygen whenever possible but may be omitted for short procedures that require less than 10 minutes of anesthesia.

Respiratory rate, and possibly heart rate, will be monitored during anesthetic procedures and recorded on a written anesthetic record sheet indicating time and duration of anesthesia, rate of isoflurane and oxygen administration, procedures performed, drugs or other products administered and reactions of the animal from induction through anesthetic recovery.

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

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

Stomach lavage - We will lavage the stomach of all sea lions captured for Task 1 except the 200 pups captured primarily for branding (see Table 1). The proper stomach lavage procedure is to first estimate the length of the stomach tube necessary by measuring the

distance to the stomach along the outside of the animal's body. The tube should be smoothly inserted into the mouth, down the left side of the animal's throat and into the stomach. To further verify that the tube is in the stomach, a small amount of air should be blown down the tube while listening for gurgling either through the tube or via a stethoscope placed on the left abdominal wall. The stomach tube will be inserted into the stomach of anesthetized animals and then gentle suction will aspirate stomach fluids up the tube, which is then pinched, extracted and the stomach contents drained into sample containers. When little or no fluid is aspirated, 1 liter of warm water can be inserted into the stomach via the tube, and then this fluid and solid stomach contents can be aspirated.

Fecal collection by enema – We will collect feces by enema from all sea lions captured for Task 1 except the 200 captured primarily for branding (see Table 1). The purpose of using enemas is to collect the contents of the digestive tract for analyses of an animal's diet. A clean, lubricated enema tube is inserted into the rectum and 1-2 liters of warm water are gently applied to flush feces from the lower digestive tract. Animals will be anesthetized with isoflurane gas during the procedure, as it would be conducted in conjunction with capture, restraint and instrument attachment or other procedures.

We are proposing to do both fecal enemas and gastric lavages on all captured sea lions > 2 months of age. They are not interchangeable techniques, nor do they provide duplicative data. Gastric lavage and rectal enemas provide different clues to suckling status and independent foraging status. Maternal milk is easily identified in gastric lavages if suckling has occurred recently, but the rectal enema cannot help identify suckling. Gastric lavage can also be used to identify recent independent feeding, but if foraging occurred more than three hours before the sampling, then the stomach is likely to be empty, but the enema will very likely show evidence of hard part remains. The hard part remains found in the lower bowel are not completely representative of all prey parts because many parts are retained within the stomach and often are eventually regurgitated. Therefore, whenever they are possible to collect, stomach contents are very valuable.

Morphometrics - Standard morphometrics including mass, length and girth will be taken from all captured sea lions. These procedures require the temporary restraint of an animal and may be partially completed when under anesthesia for other procedures. There are no adverse effects anticipated with these procedures.

Body Composition measurement – Body composition will be determined for all sea lions captured for Task 1. Body composition will be measured via three different methods. An important determinant of post-weaning survival is the energy store of the sea lion at weaning. The gold standard for determining body composition is isotopic water dilution. Two other methods of body composition analysis are bioelectric impedance analysis and ultrasonic imaging of subcutaneous blubber thickness. Although these latter two methods take less time to perform and may therefore have less impact on sea lions, we aim to increase our sample size of simultaneous measurements to properly validate the methods in Steller sea lions. Arnould (1995) has demonstrated the utility of bioelectric impedance analysis on Antarctic fur seals, but there are no published studies of its validity when used in Steller sea lions. Mellish et al. (2004) reported that

ultrasound imaging produces an accurate estimate of blubber depth, but there have not been any studies validating the use of blubber depth to predict body composition in terms of percent body fat in Steller sea lions. Therefore, we will perform all three measurements on the same individuals in order to determine whether one of the quicker techniques can eventually replace isotopic water dilution. This determination will be based upon linear regression and ANOVA analysis of the relationships between blubber depth and total body water and bioelectric impedance analysis and total body water.

Isotopic water dilution – Water enriched with stable isotopes is used to determine total body water and, by calculation, total body fat. Deuterated water (0.2 g/kg of 99.9% enriched deuterium oxide) or oxygen-18 water (~1 ml/kg for 10% enriched O-18 water) will be administered via one of the following routes: intramuscular, intraperitoneal, or intravenous. Post-injection blood samples (5.0 ml) will be collected at the following times: 1 hour post-injection and 2 hours post-injection. Sea lions will remain anesthetized during the 2 hour equilibration period.

Bioelectric impedance analysis – Electrodes (sterile needles) for bioelectric impedance analysis (BIA) will be placed subcutaneously and resistance and reactance will be recorded. Four electrodes are used in BIA, two will be placed above the skull and two near the base of the tail. Electrodes will then be removed cleaned and re-inserted up to five times to obtain repeat measurements because individual measurements of resistance and reactance can vary significantly, and therefore, it is necessary to take repeat measurements and then derive a mean value.

Ultrasound – A portable ultrasound unit (e.g., SonoSite 180Vet, as per Mellish et al. 2004) will be utilized to record blubber depth from all captured sea lions. Blubber will be measured from multiple sites for each take, including the neck, shoulder region and hind quarters. This procedure involves the application of water-soluble gel, water or alcohol to the hair, followed a momentary light pressure on the skin. This procedure is typically performed while the animal is manually restrained or during anesthesia. No adverse reactions are anticipated to this procedure.

Blood samples – All captured sea lions will be sampled for blood. Blood will be collected from the caudal gluteal, jugular, or flipper vein. Each needle may be reinserted into the skin once but only if not dull and not contaminated by handling. The size of the needle will depend on the size of the sea lion and the location of the blood withdrawal site. Each needle may be reinserted into the skin once but only if it is not dull and not contaminated by handling. A maximum of three needle insertions to initially locate a vein and subsequently obtain the required blood volume will be made at any one site at any one session to reduce muscle injury and bruising and to reduce risk of introducing infection. Blood volume collected at each take will not exceed 1 ml per kg body mass.

Milk collection from adult females – Milk collection will only occur on anesthetized adult female sea lions. Milk letdown will be stimulated by IM injection of oxytocin (0.03 ml kg^{-1}, 10 USP units ml^{-1}). Ten minutes post-oxytocin, milk will be collected manually or aided by suction from a large syringe modified by cutting the distal end so it fits over

the teat.

Dermal / mucosal swabs – Swabs will be performed on all captured sea lions to provide material for multiple assays, including epidemiology and endocrine activity. Specific analyses will include examination for bacterial, viral and other pathogens (e.g. mites, fungi, and many viruses) in addition to hormone analyses.

Skin samples – Skin samples (2 x 50 mg) will be collected from each sea lion captured. Skin will be collected using a 6 mm punch tool, either prior to flipper tag insertion or from the webbing between the hind flipper.

Whisker extraction - A vibrissa will be pulled from anesthetized sea lions >2 months old for stable isotope analysis to help identify the general trophic level at which an animal is feeding over prolonged periods. Two vibrissa (from alternate sides) will be pulled from sea lions to determine long-term diet. Pulling, rather than clipping, a vibrissae is preferable because clipping results in an unknown length remaining attached to the sea lion. Stable isotope ratios show regular, oscillating patterns in Steller's sea lion vibrissae of 1-3 cm, and changes in ratios can occur in less than 1 cm (Hirons et al. 2001). Thus, obtaining the root of the vibrissae, representing the most recent growth, for analysis is crucial. Vibrissae are pulled by gripping with forceps or fingers and pulling forcefully and rapidly in one smooth motion.

Scientific Instrument Attachment - The sea lions will be captured and then anesthetized (as described above) in order to attach the biotelemetry instruments.

For measuring the movement of a sea lion's head and flippers, data loggers that record depth, swim speed and acceleration along three axes will be attached to the head, the center of the dorsal surface and the flipper. Acceleration sensors for the head and flippers that transmit to the main datalogger are very small, cylindrical objects. They are 1.2 cm in diameter, 4.0 cm in length. Data loggers for the back measure 3 cm in diameter, 17 cm in length. During anesthesia, a baseplate (approximately 13 cm long by 8 cm wide) will be glued to the hair with epoxy. The baseplate will incorporate a remote-release mechanism so that the data loggers can be removed at the end of a field trial (typical recording periods will last between 2 days and 2 months). To directly visualize prey ingestion from a sea lion's point of view, we will utilize a recently developed, commercially produced underwater camera system, the Underwater Timed Picture Recorder (UTPR; Wild Insight Ltd, Ely, Cambs, UK). The UTPR uses a digital camera interfaced with a time-depth recorder so that the camera is activated according to preset depth criteria. The UTPR measures 10.5 x 8.5 x 5.5 cm and weighs approximately 700 g in air and 200 g in water. During anesthesia, a baseplate of the same footprint dimensions (10.5 x 8.5 cm) will be glued to the head of the sea lion with epoxy. The baseplate will incorporate a remote-release mechanism so that the UTPR can be recovered at the end of an appropriate monitoring period (typically between 12 hours and 2 weeks).

Some sea lions will be instrumented with dataloggers and sensors for recording jaw opening and closing. The actual sensor will be encapsulated in epoxy and measure approximately 1.5 cm long x 1.0 cm wide x 0.5 cm thick. It will be glued to the fur

approximately 2.0 cm from the corner of mouth, approximately 1.0 cm from inner edge of lip (see figures). The sensor will be attached to a head-mounted data logger via a 2 mm thick cable that will be glued to the fur to hold it in place. The sensor detects the distance between itself and a magnet that is placed on the lower jaw, resulting in a measure of the amount of jaw opening. The magnet will be inserted into a holder that is glued to the fur approximately 2.0 cm from the corner of mouth, approximately 1.0 cm from inner edge of lip. The magnet will be approximately 2.0 cm in diameter and 0.5 cm thick.

These newer methods of detecting prey ingestion will be compared with simultaneous measurements of stomach temperature and pressure. These variables will be monitored via an ingested stomach transmitter pill. The stomach pills are cylindrical, approximately 4.5 cm in diameter by 7.0 cm in length. The pills are housed in medical grade titanium and biocompatible epoxy. When they were housed at the Vancouver Aquarium, our captive Steller sea lions, Sugar and Kiska, were subjects in a stomach temperature telemetry development project (Andrews 1998). They ingested very similar stomach temperature transmitters and did not show any adverse affects. The stomach pills will be inserted into the stomach via a custom stomach tube.

Heart rate will be measured by attaching a heart rate data logger to the dorsal pelage (see methods for attachment of biotelemetry instruments). The heart rate datalogger will be connected via quick-release electrical connectors to two electrocardiogram (ECG) electrodes. The two electrodes will be attached subcutaneously during anesthesia to the dorsal surface in areas that gave the cleanest ECG signal for each sea lion — either one above each scapula or one above a scapula and the other above the pelvis on opposing sides. The electrodes consist of two basic parts: 1) 12 cm of 28-gauge biocompatible stainless steel cable inserted sub-cutaneously and 2) an external base of epoxy containing a quick-disconnect waterproof connector soldered to the subcutaneous cable on top of a square of neoprene glued with fast-setting cyanoacrylate to the fur of the sea lion. This method has previously been demonstrated to work safely on Steller sea lions (McPhee et al. 2003). For adult females on a rookery, we will be able to observe the sea lions directly or with the remote video system. The implant site of subcutaneous ECG electrodes will be monitored closely for any signs of irritation or infection, and if there are any complications, we will attempt to recapture the sea lion and remove the electrodes.

Two different methods of measuring breathing frequency will be used. One method involves gluing a flexible piece of stretch sensor around the chest circumference so that when the chest stretches during a breath it would be recorded by this sensor attached to a datalogger. The other method requires an air-flow sensor to be glued to the pelage near the nares. The air-flow sensor would be oblong and measure approximately 2.5 cm long by 1 cm wide and 0.5 cm tall. It would be attached to a datalogger mounted on the head via a wire that would be tacked down to the fur with epoxy. The attachment of cables to the datalogger is via a quick disconnect to prevent snagging or entanglement.
Heat flux sensors will be attached by gluing them to the fur around shaved patches of the animals' skin. The protocol will be similar to the methods developed by Kate Willis in her work on Steller sea lions at the Alaska SeaLife Center and elsewhere (Willis and Horning 2004). The heat flux sensors are flat disks that measure 2.5 cm in diameter. Sensors will be located in zones of major heat flux, such as near the neck and lower

dorsal back. Optimal locations will be determined with the aid of an infra-red thermal imaging camera. The heat flux sensors will be attached to the datalogger via short cables that can easily disconnect (in case of any snagging or entanglement).

The above instruments will be attached in different combinations, but no sea lion will receive more than this basic assortment of instruments: one head mounted instrument with a footprint of approximately 8 cm wide x 13 cm long, with a max height of 8 cm; plus a mid-dorsum mounted instrument package of similar dimensions; a stomach temperature transmitter, and in some sea lions, a third package of a satellite transmitter and VHF transmitter package that is directly glued to the fur for long-term tracking. We will determine the exact combination of instruments depending on the age and size of the sea lion, the season, the location, whether simultaneous fish assessments are occurring in the area, and whether the sea lion will be under simultaneous visual observation

A larger integrated video system/data logger, GPS and satellite and VHF transmitters (Video/Data Acquisition Platform – VDAP) will be attached to up to 20 sea lions aged 1 to 4 years and up to 20 adult females with epoxy or neoprene rubber cement (total area is ca. 0.002 square meters). After cleaning the fur in the mid-dorsal area of the sea lion with acetone, a remote release pack with the video/data recorder will be glued with epoxy or neoprene rubber cement to the fur along the dorsal midline above the shoulders. A smaller remote release system will be glued to the top of the animal's head. The combination camera head/GPS housing rests in the second release pack mounted on the head. We will use satellite transmitters (Wildlife Computers, 200 g) and VHF radio transmitters (165 MHz; 92 g; Advanced Telemetry Systems) to track and relocate instrumented animals. These transmitters will be glued with epoxy or neoprene rubber cement to the sea lion's fur. The video system will remain on the animal for up to two weeks before triggering the release. The transmitters will fall off when the animal molts.

In order to directly measure the effects of increased work demands (increased cost of swimming) that might be due to increased drag or altered buoyancy, we will equip some sea lions in the following manner: A small dive behavior data logger will be attached via a remote-release device to the mid-dorsum. Just in front of this package, a block designed to alter either the buoyancy or drag or both will be attached via a remote-release device. Blocks designed to increase buoyancy will be constructed of syntactic foam and will increase buoyancy by approximately 20 Newtons. Blocks designed to decrease buoyancy will be epoxy-coated lead and weigh no more than 2% of body mass (3 kg for a 150 kg juvenile). Control blocks that only increase drag but do not alter buoyancy will have the same exterior measurements (~12 cm high x 15 cm wide x 30 cm long) but be neutrally buoyant in seawater. All devices will be released via remote-release after no more than four weeks. Blocks can be remotely-released via radio signal if the blocks appear to be causing entanglement or other problems.

The above instruments will be attached in different combinations, and we request authorization to put any of these instruments on all of the sea lions greater than two months of age that we capture for Task 1. As indicated in the Background and Hypothesis/Objectives sections, we are planning to study sea lions aged two months to one year of age, in order to study their transition from maternal dependence to nutritional independence. The exact combinations of sensors and instruments to be deployed will be based on the age of the sea lion, the season, the location, whether simultaneous fish

assessments are occurring, whether the sea lion is under simultaneous visual observation and whether other sea lion research is occurring in the area. However, no sea lion will receive more than this basic assortment of instruments: one head mounted instrument with a footprint of approximately 8 cm wide by 13 cm long, with a max height of 8 cm; plus a mid-dorsum mounted instrument package of similar dimensions; a stomach temperature transmitter, and a third package of a satellite and VHF transmitter that is directly glued to the fur for long-term tracking. Sea lions that are instrumented with the larger VDAP camera system would not get any additional instrumentation other than a stomach temperature transmitter.

For pups < 2 months old, scientific instrumentation would not exceed the attachment of a VHF transmitter (~ 3cm wide x 6 cm long x 2 cm tall), plus a datalogging device (approximately 6 cm wide x 8 cm long x 4 cm high, or less), plus a jaw sensor (~ 1.5 cm long by 1.0 cm wide by 0.5 cm thick) and a stomach temperature transmitter.

For the buoyancy/drag experiments, we will choose from the adult females and juveniles > 2 months old listed in the Take Table, but not every sea lion will participate in these experimental manipulations. We will aim for the following sample sizes (subset of the takes in Table 1):

During the pupping season, adult females with pups < 2 mo of age will be selected for the following treatment and control groups:

Increased buoyancy: 4 - 6 adult female sea lions
Decreased buoyancy: 4 - 6 adult female sea lions
Neutral buoyancy, increased drag: 4 - 6 adult female sea lions
Control group: 4 - 6 adult female sea lions

All 4 groups will be instrumented with dataloggers; the treatment groups (increased, decreased, or neutral buoyancy) will receive drag inducing, buoyancy altering blocks, but the control group will not receive these blocks. Control group receives dataloggers only.

Outside the pupping season, adult females will be selected for the following treatment and control groups:

Increased buoyancy: 4 - 6 adult female sea lions
Decreased buoyancy: 4 - 6 adult female sea lions
Neutral buoyancy, increased drag: 4 - 6 adult female sea lions
Control group: 4 - 6 adult female sea lions

All 4 groups will be instrumented with dataloggers, the treatment groups (increased, decreased, or neutral buoyancy) will receive drag inducing, buoyancy altering blocks, but the control group will not receive these blocks. Control group receives dataloggers only.

For sea lions between 2 months and 1 year old, we will aim for the following sample size:

Increased buoyancy: 6 sea lions
Decreased buoyancy: 6 sea lions
Neutral buoyancy, increased drag: 6 sea lions
Control group: 6 sea lions

For sea lions between 1 and 4 years old, we will aim for the following sample size:

Increased buoyancy: 6 sea lions
Decreased buoyancy: 6 sea lions
Neutral buoyancy, increased drag: 6 sea lions
Control group: 6 sea lions

Temporary mark (i.e., grease stick, hair dye paint or bleach, shaved spot) – Because our research plans sometimes require us to capture pups before they have reached the ideal age for branding, we will need to mark them in a temporary manner. We prefer to brand pups once they are greater than 2 weeks of age because by that time they will have developed a stronger immune system and be more likely to heal successfully. Those that are physically captured will be marked by fur clipping or with human hair bleach. However, in order to determine which pups belong to which mothers, and therefore the age of the pups before they are captured, it would be less invasive if we could mark them remotely and then capture only those individuals that were the best candidates for the study. This will also give us a larger cohort of identifiable pups in order to monitor their time activity budgets and survival rates. Therefore, we request permission to mark pups with paint or hair bleach or by clipping fur. When necessary, sea lions will be marked without capture by projecting paint balls filled with paint or hair bleach onto the middle of the torso. This would be required, for example, when we wish to capture a mother/pup pair that is surrounded by other pups that might be confused with the target pup. By first marking the pup and then darting the female, we can be certain to identify the correct pup. The projection of balls will be from a distance of between 2 and 10 meters. The paint ball projection devices were designed for marking livestock and have been shown to be safe for marking animals. Pups that will be captured for biological sampling but are too young to brand will be marked with an easily identifiable alpha-numeric mark by clipping a small amount of fur with battery-powered clippers and/or marking fur with hair bleach. If a small number of pups are marked on a rookery, simple bleach marks can provide a sufficient number of unique marks, but when larger numbers of pups must be marked, it is easier to make unique alpha-numeric marks by clipping the fur.

Hot brand – Hot branding will be conducted on Steller sea lions as described in Merrick et al. (1996). We will aim to hot brand all of the Steller sea lions that we capture, unless it is deemed that the temporary (< 2 week) inflammatory response (Mellish et al. in press) that occurs might affect an important parameter that is being measured with telemetry instruments. In most of those cases, we will be aiming to recapture individuals (to retrieve instruments, measure changes in morphometrics and take biological samples), which will be hot-branded at that time. Hot brands will place permanent, unique numbers and/or letters on the animal's left flank to improve the ability to identify the animal. Only qualified veterinarians or other personnel with sufficient experience in the technique will be allowed to perform this procedure. Hot irons will only be applied long enough to permanently mark the superficial dermis and not damage the underlying tissue (approximately 3-4 sec). Branding irons are made from 5/16" cold-rolled, stainless steel round-stock and are approximately 2" wide by 3" tall. Brands are applied while the animal is under anesthesia in order to minimize pain. There is the potential for infection at the wound site, and the activity of the animal may prolong or prevent healing by producing repetitive stress on the wound. However, individuals typically appear to respond well to the procedure and show no clinical signs of infection or inflammation within weeks (Mellish et al. in press). Other agencies currently branding Steller sea lions (pups and juveniles) are the National Marine Mammal Laboratory, WA and the Alaska Department of Fish and Game. Concerted efforts for brand-resight information occur

between these two agencies and ASLC to monitor survival, natality rates and age at first reproduction. Given the increased amount of scientific activity with Steller sea lions, it is imperative to know if an animal has been captured previously in order to obtain the maximum amount of information in the long term for each animal.

Flipper Tagging – Sea lions that are not branded on their first capture event will be marked with plastic cattle ear tags for future identification. These tags are not as easily readable as large bleach marks, but if for some reason it is not possible to recapture and hot-brand a sea lion before it molts that year, they can provide a mark that last past the molt, allowing potentially long-term identification of a previously studied sea lion. These tags will be affixed through the foreflipper in loose skin posteriorly, near the area where the flipper meets the body. The hole is made with a punch. Each animal receives two tags, one per flipper, to minimize the chance of losing the ability to identify the animal should one tag be lost.

Tissue collection (blubber, skin and muscle biopsy) – We will collect blubber, skin and muscle biopsies from all sea lions captured for Task 1 except the 200 pups captured primarily for branding (see Table 1). We will collect skin, blubber and muscle biopsies from sea lions for enzyme, fatty acid and contaminant analysis. Skin samples will be used for genetic and contaminant analyses. Blubber will be used for stable isotope and fatty acid analyses. Muscle will be used for energetic and enzyme analyses. Enzyme profiles will be examined to determine the aerobic condition of sea lions. The aerobic condition of sea lions may be an important determinant of their diving ability and therefore foraging success and ultimate survival. Stable isotope and fatty acid profiles will provide an indication of prey selection, which may also be an important factor in the condition and survival. Contaminant loads are another factor that we will examine for correlations with the ability to forage successfully and survive to reproduce.

We will take biopsies from either the base of the neck or dorsal loin region. The exact site will be chosen at the time of sampling depending on whether there are previous wounds in one are or the other. A sterile scalpel will be used to make a small incision to collect skin and make an opening for a biopsy punch. The sterile biopsy punch (5.0 ml diameter) will be used to collect up to 2.0 g of blubber and 0.5 g of muscle. The blubber and muscle will be removed from the same incision site. The maximum depth of each biopsy sample will be approximately 2.0 cm below the skin. The small wound will be left open to heal on its own.

Tooth Extraction – We will collect a tooth from all captured sea lions greater than two months of age. Age is a critical component to all physiological interpretations and foraging patterns, as well as population structure and distribution. Current methods that are utilized to determine age include patterns of body mass and morphometrics and tooth eruption. While we are fairly accurate with these methods in the first few months of life, it is recognized by agencies such as ADF&G and NMML that these methods are inaccurate for older animals. Pulling a tooth allows examination of tooth characteristics including size, root closure, incremental cementum growth layers, dentine thickness and crown appearance, which when combined with eruption patterns, mass and body length permits much more accurate age estimation. The method used would follow that

currently utilized by ADF&G and NMML, in which a dental elevator is used to loosen the tooth attachments and extraction the tooth. Sea lions would be anesthetized and the area around the tooth cleaned before extraction. The dental elevator will be sterilized before using on each sea lion. If necessary, after extraction, pressure will be applied to the cavity until bleeding has stopped. Only one premolar tooth will be removed for analysis.

Photo-identification – Images of individuals of all ages will be captured using the remote video system or digital photography in the field for the purposes of photo-identification. This includes branded, tagged, or otherwise marked by researchers, as well as naturally marked individuals that may have unique flippers, scars, fungal patches, etc. Small vessels 18–25 feet in length may be used for dedicated brand/tag-resight surveys. Multiple (≥ 4) approaches < 100 meters from rookeries and haulouts will be necessary, while every attempt is made to keep disturbance at a minimum. There are no adverse effects anticipated with these procedures with the exception of incidental disturbances.

Material Collection – Naturally excreted fecal material, placentas, aborted fetuses and dead individuals will be collected opportunistically. Fecal samples will be shared among tasks to be examined for prey remains, vitamin content, hormones and evidence of parasitic infection. Expelled placentas will be collected for examination and will follow protocols outlined in Appendix B (ASLC Carcass/Tissue Handling Protocol). Placentas will be examined for basic structure and function, enzymology, contaminants, immunoglobins and endocrinology. They will also be tested for the presence of *Brucella, Leptospira, Chlamydia, Herpes* and other infectious organisms. Aborted fetuses will be collected opportunistically to be tested for the presence of *Brucella, Leptospira, Chlamydia, Herpes* and other infectious organisms. These fetuses will also be examined for contaminant load. Deceased newborns (up to one month of age) will be collected annually opportunistically to be tested for the presence of *Brucella, Leptospira, Chlamydia, Herpes* and other infectious organisms. These neonatal carcasses will also be examined for contaminant load. Major organs (e.g., lungs, kidneys, heart, liver, brain, eyes) of freshly dead animals will be collected opportunistically and analyzed for contaminant load. Tissues that are not used within the scope of this permit application will be made available to other researchers through the Alaska Regional Stranding Network. There are no anticipated adverse effects associated with these procedures with the exception of incidental disturbance. Collection of these materials will occur during other research activities such as scat collection or sea lion capture.

Scat will also be collected from rookeries at various sites in the Gulf of Alaska and the Aleutian Islands. A site will not be visited for scat collection more than four times annually, with no less than three days separating collection dates. Previous studies (Mashburn and Atkinson 2004, 2006) have indicated that adrenal response to a perceived stressor is cleared in feces with 52 hours; therefore, separating collection dates by at least three days removes the possibility that high corticosterone concentrations are due to the presence of researchers collecting scats. We will coordinate with all other permitted Steller sea lion researchers so that researcher presence on a site is known. To ensure the most comprehensive representation of stress hormone concentrations, all scat visualized

on each rookery will be collected, assigned an identification number and relevant data regarding conditions at the collection sites (i.e. recent storms or orca activity) noted. Following their return to the laboratory, samples will be processed, sexed and assayed according to methods reported by Mashburn and Atkinson (2004). Resultant values will then be analyzed to determine whether the animals on rookeries closest to Unalaska exhibit stress hormone concentrations chronically higher than those animals on surrounding rookeries. Excreted fecal material will also be collected from rookery and haulout areas at times when animals are being disturbed for other reasons (e.g. animal capture). Feces will also be collected with a remote-controlled vehicle equipped with a robotic arm. The remote-controlled vehicle will be battery powered and very quiet. It will be camouflaged so that it looks like a rock. It will be designed to act like a turtle, in that its external shell can be lowered down to the substrate. In effect, the vehicle will "hunker down" when not moving. It will be controlled by a hidden operator.

Task 2

Table 2 outlines all proposed activities for free-ranging Steller sea lions, including capture, restraint, morphometrics, digital imaging, blood and tissue sampling, deuterium oxide dilution, ultrasound, fecal and urine collection, whisker extraction, skin/mucosal swab, hair collection, hot-brand, metabolic chamber and scientific tag attachment. Of these individuals, up to 30 juveniles per year may be included in the Transient Juvenile Steller Sea Lion Project. Takes for these individuals during their temporary captivity period are listed separately in **Table 3** for clarity, with additional takes of temporary captivity, x-ray, dry holding, controlled fasting and LHX implantation. Detailed descriptions of each procedure are shown below listed in alphabetical order. Procedures denoted with an asterisk (*) will take place on temporarily captive transient juveniles only. For all procedures listed, activities will only be performed by/under the direct supervision of qualified and experienced personnel. An emergency kit with equipment and supplies for responding to complications will be readily available. Whenever possible, most procedures, including but not limited to blood and tissue collection, whisker extraction, hot-branding, attachment of scientific instruments and x-ray, will occur while the animal is under general anesthesia in order to reduce potential stress to the animal as well as increase the safety of the individual and the handlers.

Attachment of scientific instruments – Satellite-linked dive recorders (approximate dimensions 10cm x 4cm x 4cm) will be attached to captured pups and juveniles and transient juveniles prior to release. The animal will be anesthetized with isoflurane and respiration will be closely monitored. The attending veterinarian(s) will be present during all procedures for this study to monitor the physiologic state of each animal. Dive recorders will be attached to the dorsal pelage with five-minute epoxy to record and transmit movement and dive information until the battery is expended or until tag release during the annual molt. There are no adverse effects anticipated with these procedures.

Up to five transient juveniles per year will also be outfitted with a video system/data logger and transmitters (satellite and VHF) via epoxy or neoprene rubber cement attachment (total area is ca. 0.002 square meters). After cleaning the fur in the mid-dorsal area of the sea lion with acetone, a remote release pack with the video/data

recorder will be glued with epoxy or neoprene rubber cement to the fur along the dorsal midline above the shoulders. The acetone and volatile components of the neoprene cement may be absorbed into the skin. We use a slow-setting epoxy to minimize the chance of burning the skin. Since the acetone is applied to a sponge to clean the fur, little comes into contact with the skin, and no adverse reactions are expected. Likewise, only small amounts of epoxy and neoprene rubber cement are applied to the fur. A smaller remote release system will be glued to the top of the animal's head to hold the video camera. Once mounted on the animal, the entire video system/data recorder is neutrally buoyant in water and weighs about 2 kg in air (about 1% of the mass of a juvenile and 0.7% of the mass of an adult female SSL). The instrument will augment hydrodynamic drag, but the cross sectional area of the instrument will be less than 2% of the maximum cross sectional area of the animal, so drag augmentation will not be large. The dual release system can be remotely triggered to allow the video system/data recorder to fall off. The low-profile, remote release pack eventually falls off when the sea lion molts three to six months later. We will use satellite transmitters (Wildlife Computers, 200 g) and VHF radio transmitters (165 MHz; 92 g; Advanced Telemetry Systems) to track and relocate instrumented animals. These transmitters will be glued with epoxy or neoprene rubber cement to the sea lion's fur. The video system will remain on the animal for up to two weeks before triggering the release. The transmitters will fall off when the animal molts three to six months later. Based on previous studies of this instrumentation with Weddell seals, the video/data recorder does not appear to diminish foraging and prey capture (Davis et al. 1999).

Blood sample – All pups and juveniles captured will be sampled for blood. Transient juveniles will be sampled for blood up to twelve times prior to release. Blood will be collected from the caudal gluteal vein or flipper vein, depending on the volume of blood required and accessibility of veins at the time of sampling. Each needle may be reinserted into the skin once but only if it is not dull and not contaminated by handling. A maximum of three needle insertions to initially locate a vein and subsequently obtain the required blood volume will be made at any one site at any one session to reduce muscle injury and bruising and to reduce risk of introducing infection. Blood volume collected at each take will vary dependent upon the specific project requirements for each individual but will not exceed the lesser of: a) 1 cc per kg body mass or b) 5% of total blood volume per month (based on animal mass at the time of collection, as per Murray 2000). A catheter will be used when multiple samples (> 5) must be collected over time (e.g., tracer equilibration curves) in order to reduce the number of needle insertions required. There is a small risk of infection associated with penetration of the animal's dermis by the needle. Multiple attempts to obtain a blood sample are stressful and may cause some degree of pain, damage to the vein, clotting, bruising and abscess. To reduce the risk of infection, only clean, sterile disposable needles will be used to obtain blood samples, and a new needle will be used for each blood collection.

Capture/restraint – We will capture Steller sea lion pups and juveniles on land using hoop nets or underwater via lasso, as is standard procedure by the National Marine Mammal Lab, Alaska Department of Fish and Game and ASLC for animals of this age class (Merrick et al. 1997; Mellish et al. 2006). This technique has proven to be effective and safe for divers and captured animals. The underwater lasso method is preferred and

will be used when weather conditions are amenable to diver immersion and sea lion retrieval. In the event that it is deemed safe and preferable by the Chief Scientist to perform a land capture, the hoop net will be employed. In addition, we will utilize the platform capture method, which typically employs a 12-ft. wide buoy with a 12-ft. by 12-ft. platform for a haul-out surface. There are 6-ft. high steel cage walls around the perimeter of the platform with a wide trap door on one side. Sea lions haul out and return to the water freely through the trap door. To capture sea lions, the trap door is dropped when sea lions are hauled out inside. Captured sea lions are transferred into a holding cage on a 30-foot barge that docks with the capture cage; then, they are moved one at a time from the holding cage into a stainless steel squeeze cage. This system is analogous to handling runs and squeeze cages used for livestock on farms and ranches. The squeeze cage restricts the movement of the sea lions without harming them or exposing handlers to unnecessary risk. While an individual sea lion is in the squeeze cage, we can weigh and measure it, attach satellite-linked depth recording instruments, apply brands or tags, draw blood and collect other biological samples. When all procedures are completed, the squeeze is opened, the animal is released and the next animal is allowed in.

If deemed necessary by the attending veterinarian, anesthesia will be administered to facilitate sampling procedures. Whenever possible (e.g., captive environment), food will be withheld for 12 hours prior to general anesthesia. Animals that can be positioned in sternal recumbency and restrained by use of the squeeze cage bars or a capture box will be masked with isoflurane gas anesthesia using a modified traffic cone held tightly over the face to create an air seal. Additional restraint of the head will be achieved by using a head board and by placing bumper pads on either side of the head under the squeeze bars to assist in positioning nose forward for placement of the mask. Animals that cannot be adequately restrained for mask induction will be premedicated with intramuscular injections of atropine (0.54 mg/ml) dosed at 0.03 ml /kg and followed at least 10 minutes later by Telazol (tiletamine HCl and zolazepam HCl) at a dose of 0.5 -2 mg/kg. Midazolam will be used as an alternate drug for sedation at a dose of 0.2 mg/kg IM. This dose does not immobilize but does appear to reduce anxiety and resistance to manipulation therefore allowing use of the squeeze cage restraint for administration of the isoflurane mask induction. It is possible to reverse some effects of midazolam with an injection of flumazenil (1 ml / 10-15 mg midazolam IV or IM). The sedation/preanaesthetic drugs are administered intramuscularly by jab pole syringe. Depth of anesthesia will be judged by the anesthetist based on respiratory rate and volume, response to stimuli, palprebral reflex, capillary refill and jaw and muscle tone and maintained using 1% to 3% isoflurane in 5-10 liters of oxygen per minute flow rate as needed. Intubation with an appropriate sized (10-16 mm) cuffed endotracheal tube should be utilized for continued administration of isoflurane gas and oxygen whenever possible but may be omitted for very short procedures (less than 10 minutes) or when otherwise contraindicated.

Hypothermia (deep rectal temperature < 92 ° F) will be prevented by the application of warm (100-105 °F) water bags to flippers and body, drying of the fur and covering the animal with thermal insulating blankets. Hyperthermia (deep rectal temperature > 106°F) can be controlled by wetting the flippers with cool water, applying ice or cold water packs and use of fans for increased air flow. An emergency kit will be present at all times consisting of a respiratory stimulant (e.g., doxepram), a cardiac stimulant (e.g.,

epinephrine), a parasympatholytic agent (e.g., atropine) and a corticosteroid (e.g., dexamethasone). Positive pressure oxygen ventilation utilizing the endotracheal tube and a rebreathing bag or an assisted respiration bellows system on the anesthetic machine will also be available if needed. Administration of isoflurane gas will be discontinued as soon as possible after the completion of necessary research procedures. Oxygen will be administered for several additional minutes until the endotracheal tube can be removed (as judged by the return of jaw tone and swallowing reflexes). The animal will be monitored by the anesthetist for vital signs and body temperature until it regains voluntary mobility.

Deuterium oxide dilution – Deuterium oxide (D_2O) will be administered to non-lethally evaluate body composition via isotope dilution methodology once per captured pup and juvenile and up to four times for transient juveniles to monitor body condition during temporary captivity. Pre-sterilized, pre-weighed D_2O will be administered intramuscularly or intravenously. An initial blood sample (10 cc) is drawn prior to tracer administration for reference. Two post-injection samples (10 cc each) are collected at approximately 120 and 135 min to ensure full equilibration of the tracer in the body water pool. Animals may be maintained under anesthesia for the duration of the equilibration period or manually restrained via squeeze cage for post-D_2O blood samples.

Digital imaging – Digital imaging, including thermal imaging, will take place on all captured pups and juveniles while the animal is under anesthesia or on an opportunistic basis. There are no adverse effects anticipated with these procedures.

Dry holding* – Animals will be held in outdoor dry runs or metabolic/transport cages for up to 48 hours to facilitate metabolic studies, urine and fecal sampling. Each transient juvenile will undergo up to two dry holding events during temporary captivity, not including transport to and from the facility during capture and release events. Animals will be held in either a dry run or holding cage measuring approximately 7’ long x 5’ high x 3’ wide, which will adequately confine the individual to allow for sample collection but not overly restrict movement.

Fecal/urine collection – Excreted urine and fecal material will be opportunistically collected from the holding area of all individuals. There are no adverse effects anticipated with these procedures.

Hair clipping – A small amount of hair for contaminants analysis will be collected from all individuals during temporary marking procedures (e.g., clipper mark). There are no adverse effects anticipated with these procedures.

Hot-brand – Hot branding will be conducted as described in detail in Mellish et al. (in press). Hot irons will only be applied long enough as determined by rate of removal of the hair and outer cell layers to permanently mark the superficial dermis and not damage the underlying tissue. Brands are applied while the animal is under anesthesia in order to minimize pain. There is the potential for infection at the wound site, and the activity of the animal may prolong or prevent healing by producing repetitive stress on the wound.

However, individuals typically appear to respond well to the procedure and show no clinical signs of infection or inflammation within weeks (Mellish et al. in press). Other agencies currently branding animals in Alaska (pups and juveniles) are the National Marine Mammal Laboratory, WA and the Alaska Department of Fish and Game. Concerted efforts for brand-resight information occur with these two agencies to monitor survival, natality rates and age at first reproduction. Given the increased amount of scientific activity with Steller sea lions, it is imperative to know if an animal has been captured previously in order to obtain the maximum amount of information in the long-term for each animal.

LHX life history transmitter implantation* – Implantation of dual life history transmitters will be performed with a minimum of three people: a surgeon, an anesthetist and a non sterile surgical assistant. Standard aseptic surgical technique will be practiced, including an appropriate cap and mask and a sterile barrier surgical gown and gloves. The following includes a template protocol for the surgery; however, this method is still in development and may be modified to minimize the effects on the animal. The surgical site will be prepared by clipping hair, skin disinfection and the use of a sterile drape. The transmitters will be gas-sterilized utilizing ethylene oxide gas (EO) in suitable packaging permeable to gas but not to bacteria. Gas sterilized transmitters should be allowed to outgas for a period of 24 hours before implantation. Surgical instruments and moisture barrier surgical drapes will be purchased pre-sterilized or thoroughly washed, dried, packaged and sterilized in an autoclave or by EO gas. The animal will be positioned securely on the surgical table in dorsal recumbency. A warm water flow under the table and thermal insulating pads covering the surgical table will retard heat loss. Anesthesia will be monitored by use of a respiratory or cardiac monitor. The surgical site will be between the caudal sternum manubrium and the pubic bones, palpated through the abdominal wall. An area 8-10 cm long and 4 cm on either side of the midline should be clipped and hair removed. The skin will be repeatedly scrubbed with alcohol (90% isopropyl) alternated with povidone iodine on clean gauze sponges. A nonporous sterile fenestrated drape will be placed over the surgical site and held with towel clamps.

The skin will be incised along the ventral midline; the subcutaneous layer and blubber are sharp dissected. The *linea alba* will be lifted with forceps to permit penetration of the abdominal wall with a scalpel blade. The *linea alba* is then sharp dissected with blade or scissors, avoiding the viscera, to a length sufficient to pass the transmitter body (approximately 7-8 cm). Alternately, as determined by the attending veterinarian and particular condition of the individual, a skin incision will be made parallel to the long axis of the body in the paralumbar fossa (ventral to the lumbosacral muscles and anterior to the origin of the sartorious muscle). The incision should be extended with sharp dissection through the subcutaneous and blubber layers and through the superficial layer of the lumbodorsal fascia. When the muscular abdominal wall (transverse abdominal muscle) is reached, the fascial layer should be incised parallel to the muscle fibers for 1 to 2 cm and blunt dissection used to enlarge the opening through the muscle and peritoneal layers sufficiently to insert the transmitter (diameter 5.5 cm). The abdominal wall will be grasped on either side of the incision with tissue hooks and lifted up and laterally while the transmitter is inserted through the incision into the abdominal cavity or a tapered trochar will be used to dilate the peritoneal opening and

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

Animals will be monitored at all times by a team of qualified veterinarians. As outlined in the procedures above, all attempts to maintain a clean, aseptic and whenever possible, sterile environment will be made. Only qualified veterinarians or other personnel with sufficient experience (e.g., Wildlife Biologists with > 5years of surgical experience) in the technique will be allowed to perform this procedure. An emergency kit with equipment and supplies for responding to complications or emergencies will be readily available. Any animal displaying evidence of infection (swelling, wound discharge, changes in appetite) will be treated with antibiotics or additional surgery as needed and recommended by the attending veterinarian.

Metabolic chamber – The chamber is designed to completely enclose an occupied sea lion capture box (described above in **Capture**). There are 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. In a small number of cases, we will measure the metabolic rate of animals during the short waiting period prior to initial anesthesia, then, subsequently, while they are resting on deck between initial anesthesia and injection of deuterium and throughout the final anesthesia period during which the remainder of samples are collected. This part of the study is to verify that there is no alteration of the metabolic rate resulting from the anesthesia procedure itself. This will require a maximum of two metabolic chamber collections in one capture period for these animals. During 2003, Dr. Rea (CI) conducted this experiment on 23 wild-caught animals with no noticeable effects, such that no adverse effects are anticipated and no mitigation is required.

Morphometrics – Standard morphometrics will be taken from all pups and juveniles, including mass, length and girth. Morphometrics will be taken up to weekly from transient juveniles. These procedures require the temporary restraint of an animal but, otherwise, have no adverse effects on the individual. There are no adverse effects anticipated with these procedures.

Skin/mucosal swab – Swabs will be obtained for multiple physiological studies and health diagnostics from each captured pup and juvenile. Transient juveniles will be swabbed at any point during their temporary captivity as deemed necessary by the

attending veterinarian and PI for diagnostic purposes. There is a very small risk of infection associated with swabbing the animal's dermis, rectum and ocular area. To reduce the risk of infection, only sterile disposable swabs will be used.

Temporary captivity* – Up to 30 juveniles per year will be selected for temporary captivity at the South Beach facility at ASLC. There will be up to six individuals in temporary captivity at any given time pending the specific research projects scheduled per cohort. Typically, cohorts of 4-6 individuals will be housed at the facility. Animals will only be held on an individual basis for a specific research need or under exceptional circumstances in which the individual would be a danger to the remainder of the cohort or vice versa (e.g., health condition, injury, or extreme aggressive nature). Individuals may be held for up to a maximum of three months. Details of the facility, husbandry and sanitation procedures are outlined in Mellish et al. (2006).

Temporary mark (i.e., grease stick, hair dye, shaved spot) – All pups and juvenile animals will be marked temporarily with a commercial cattle marker/grease stick, hair dye or shaved spot as appropriate for temporary individual identification. Temporary marks are used in addition to the branding process to facilitate remote identification prior to branding as well as to allow for identification from all sides of the individual (e.g., when animal is reclined on branded side).

Tissue collection – This will include blubber (≤ 200mg), muscle (≤ 200mg) or skin (≤ 50mg). One biopsy of each type may be collected from each pup and juvenile collected. Transient juveniles will be sampled for blubber up to four times during the temporary captivity period. Personnel involved in biopsy should be identified prior to each activity and must have completed appropriate training and orientation in animal use and care, safety, animal restraint, sterile technique, biopsy procedure and sample handling (i.e., present at a minimum of five prior events and directly supervised by an experienced staff member). All instruments, gauze sponges and scalpels will be sterilized by steam autoclave or ethylene oxide methods or purchased pre-sterilized prior to each biopsy. All instruments and materials will be replaced or re-sterilized between each biopsy session or if inadvertently contaminated during a procedure. The skin is cleansed with isopropyl alcohol and dilute povidone iodine/sterile saline solution. An incision will be made through the skin approximately 1.5-2 cm long. The wound is left open to heal, as it has been indicated by ASLC veterinary staff that no problems with post-operative infections have been noted in Steller sea lions with this procedure. Animals will be held in a cool, dry area until completely recovered from anesthesia and monitored for signs of bleeding or discomfort. There is a small risk of infection associated with penetration of the animal's dermis with a scalpel/biopsy punch tool to obtain subcutaneous blubber. To reduce the risk of infection, only clean, sterile disposable scalpel blades or biopsy punch tool will be used to obtain biopsy samples and a new scalpel blade or biopsy punch tool will be used for each biopsy site.

Ultrasound – A portable ultrasound unit (e.g., SonoSite 180Vet, as per Mellish et al. 2004) will be utilized to record blubber depth from all captured pups and juveniles, and up to weekly from transient juveniles. Blubber will be measured from multiple sites for

each take, including the neck, shoulder region and hind quarters. This procedure involves the application of water-soluble gel, water or alcohol to the hair, followed by momentary light pressure on the skin. This procedure is typically performed while the animal is manually restrained via squeeze cage or during anesthesia. No adverse reactions are anticipated to this procedure.

Whisker extraction – A vibrissa will be pulled from anesthetized sea lions > 2 months old for stable isotope analysis to help identify the general trophic level at which an animal is feeding over prolonged periods. Two vibrissa (from alternate sides) will be pulled from transient animals to determine changes due to diet during the captivity period. Pulling, rather than clipping, a vibrissae is preferable because clipping results in an unknown length remaining attached to the sea lion. Stable isotope ratios show regular, oscillating patterns in Steller sea lion's vibrissae of 1-3 cm, and changes in ratios can occur in less than 1 cm (Hirons et al. 2001). Thus, obtaining the root of the vibrissae, representing the most recent growth, for analysis is crucial. Vibrissae are pulled by gripping with forceps or fingers and pulling forcefully and rapidly in one smooth motion.

Fasting* – Of the 30 animals per year selected for the Transient Project, up to 12 will undergo temporary partial or full food restriction of up to 10 days. Specific selection will be determined by season, sex and capture mass. Each of the 12 individuals will undergo up to two fasting events with a minimum two week buffer interval. The number of fasts per animal will be pending the total research load, the time spent in temporary captivity and satisfactory response to an initial fast (i.e., total body mass after recovery period is equal to or greater than 100 kg). This procedure will also be combined with pre- and post-fast blood collection, metabolic chamber, digital imaging, D_2O administration, ultrasound, morphometrics and blubber biopsy. Studies of juvenile Steller sea lions have shown no overt detrimental effect of temporary fasting of up to two weeks (Mellish and Horning, in review). Metabolic chamber readings of basal metabolic rate and blood samples will be collected up to four times during the fasting period (manually restrained via squeeze cage or while under anesthesia) for evidence of fasting metabolites and hydration status.

X-ray* – A mobile x-ray unit will be used to examine transient juveniles, including all extremities, the head and neck, chest, abdomen (anterior and mid) and the pelvis as deemed necessary by the attending veterinarian and Principal Investigator, up to twice per individual. This procedure requires that the animal be under anesthesia. The primary discomfort associated with this procedure is immobilization, which is eliminated with the use of anesthesia.

3. Additional Information for Removing Animals from the Wild into Captivity and Research or Enhancement on Captive or Rehabilitating Animals
This section applies to Task 2 only.
a. Removing animals from the wild/research on captive animals:
Given the unfeasibility of multiple recaptures of free-range animals, the utilization of individuals held for short-term observation will provide the most accurate information possible. In order to collect accurate information to assist the recovery effort for the

Steller sea lion stock, the population in question must be monitored and studied. The use of alternate species, captive or free-range, would provide data of limited usefulness. The existing captive Steller sea lion population in North America consists of a few resident individuals at the Alaska SeaLife Center (Alaska), Mystic Aquarium (Connecticut) and the Vancouver Aquarium (Canada). There are no currently identified individuals from the Western U.S. stock in long-term captivity. Physiological studies to be conducted during the temporary captive period and behavioral studies post-release are not feasible on long-term captive animals. The utilization of free-ranging animals maintained in captivity for a minimal period of time (i.e., ≤ 3 months) will be essential for accurate data collection, as long-term handling and residency may influence even basic hormonal and clinical parameters. The short-term captive period will allow for sufficient time to conduct research without long-term influences on physiology or behavior. In addition, studies conducted on free-ranging animals will provide essential data pertaining to the stock as it exists in its present state, not altered or influenced by the constraints of long-term or short-term captivity.

b. Provide a description of the enclosure to be used for containment and the transport, mode of transportation, special care during transport, and the length of time required for the transfer from the capture site to the initial holding facility, and then to the permanent holding facility.

Juvenile Steller sea lions (1-4 years of age) will be captured in water or on land using standard techniques as described in detail under **Methods** and **Capture**. Animals will be maintained temporarily (≤ 4hrs) in capture boxes similar to those currently in use by ADF&G and NMML (e.g., dimensions approximately 69" long x 15" high x 24" wide). Animals will be maintained in these capture boxes until either immediate transport to the ASLC facility or the support research vessel. For non-immediate transfer via support vessel (i.e., transport time of up to 48 hours), animals will be held in specialized holding containers (7' long x 5' high x 3' wide). A qualified member of the research or husbandry team will be assigned to monitor the temperature and overall environment of the transport box for the duration of the holding period. Animals will be sheltered from noise and visual disturbance as much as possible. A veterinarian will be available at the point of departure and arrival. An emergency kit will accompany the animal(s) at all times.

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

Not applicable.

(d) If the source stock is from marine mammals already in captivity (other than beached/stranded animals) indicate the name and location of the facility, and identify the specific animals involved in the proposed activity.

Not applicable.

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

A copy of the most recent APHIS inspection report is attached.

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

A copy of the requested statement is attached.

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

The South Beach Facility at ASLC was designed specifically for the temporary housing of juvenile Steller sea lions for the purposes of research. Details of construction, sanitary and husbandry procedures are available in Mellish et al. 2006. Diet will vary pending research requirements but will typically include known prey items of Steller sea lions, such as pollock and herring. The husbandry staff at ASLC has multiple years of experience with captive marine mammals and three years of experience specific to the South Beach facility. Over the course of the first three years, the husbandry staff have developed methods specifically for the management of temporarily captive individuals (Christen et al. 2005).

(h) Indicate whether a captive breeding program will be established.

Not applicable.

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

Animals will be released to the wild at the termination of research activities at known haul-out locations or with conspecifics within the known range for the population.

(j) *If release of captive marine mammals to the wild is proposed, state the length of time the animals will be held, no matter how temporary, and describe the protocols for the release, including post-release monitoring protocols. Include in the release protocol mitigation for the following:*
-disease transmission between released animals and the wild population;

Each group of captive transient animals will be transported and maintained at ASLC for periods of up to three months. Individuals will be maintained in a specially designated quarantine area (described in Mellish et al. 2006) with dedicated husbandry staff to eliminate any potential disease transmission between the resident and transient individuals. All transient individuals will undergo a comprehensive health screening at the time of capture and again prior to release. Due to the strict quarantine housing conditions, any disease condition found upon release screening can be assumed to be pre-existing and therefore not a health threat to the wild population. Although rare, disease screening protocols do include a miniscule rate of missed or incorrect identification.

- *potential genetic exchanges between introduced and endemic stocks*;

Transients will be released only in the immediate region of their original location, preventing any artificial mixing of genetic stocks.

- *ability of the released animals to forage and protect themselves from predators;*

Only animals greater than one year of age, as determined by a combination of time of year, body mass and tooth eruption patterns and most likely weaned, will be selected for the transient program. Therefore, transients will have developed adequate foraging and predator avoidance behaviors prior to capture. All efforts will be taken to minimize exposure to humans. The use of live prey, and/or a mix of live and dead prey will be utilized whenever feasible. The brief period of holding at ASLC should minimize any loss of these behaviors. Preliminary data from released juveniles indicate no impediment to normal foraging behaviors (Schrader et al. 2004; Thomton, Horning & Mellish, unpublished data).

- *elimination of behavioral patterns acquired during captivity that could prove detrimental to the released animals or the social structure of local populations*.

Short-term captive transient juveniles will only be housed with other transients, eliminating any learned behavior from the resident, captive animals. In addition, the transient juveniles are visually quarantined from the long-term captive Steller sea lions as well as from human presence to the greatest extent possible. The limited time period the animals are to be held will minimize any alterations in their normal or pre-existing behaviors. Due to the limited human contact and remote feeding system utilized, any sea lion behaviors learned or modified due to interactions with the husbandry and research staff will be extremely context-specific and not likely transferable to the wild environment, as discussed in Mellish et al. 2006. Satellite data from released transient animals indicate no alteration to normal foraging behaviors (Schrader et al. 2004; Thomton, Horning and Mellish unpublished data), and opportunistic sightings of released animals on remote video systems have revealed no abnormal social behavior post-release.

4. Lethal take

Tasks 1 & 2

a. There are no intentional lethal takes requested.

b. Unintentional mortality or serious injury:

There is no intentional lethal take in conjunction with any aspect of this application. We request authorization for the unintentional mortality and/or euthanasia of up to seven animals annually for this permit, including the free-range and captive transient program component at ASLC. For temporarily captive animals, in the event that an animal passes the health screen at the onset of the project but does not meet these criteria upon the final health examination and is deemed a risk to the free-ranging population, we request authorization for extended holding for treatment and/or euthanasia pending the severity of the condition. The duration of extended holding would be determined on a case-by-case basis as determined by the Attending Veterinarian and Chief Scientist with consideration of the individual condition and prognosis for recovery and release. In the

event that more than two animals are deemed non-releasable within the period of one year, we will review and re-evaluate the process.

Should euthanasia be necessary, we will follow protocols already in place (Appendix C). Tissue collection and carcass disposition for incidental deaths will follow carcass collection protocols (Appendix B).

5. Exports of Marine Mammals from the U.S.

No export of live individuals or animal parts is requested.

D. Research Effects and Mitigation Measures

Tasks 1 & 2

1. Effects

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

Attachment of scientific instruments – There are no anticipated risks associated with satellite tag attachment. VDAP instruments will augment hydrodynamic drag, but the cross sectional area of the instrument will be less than 2% of the maximum cross sectional area of the animal, so drag augmentation will not be large. Based on previous studies of this instrumentation with Weddell seals, the video/data recorder does not appear to diminish foraging and prey capture (Davis et al. 1999). Attachment of foraging sensors to the mouth area could cause irritation of the sea lion, but similar sensors attached to seabirds have been well tolerated and apparently do not impede prey capture (Wilson et al. 2002). Since acetone is applied to a sponge to clean the fur, little comes into contact with the skin and no adverse reactions are expected. Likewise, only small amounts of epoxy and neoprene rubber cement are applied to the fur. Sea lions typically ignore the instruments and do not attempt to bite or rub them off, and they usually begin foraging within one day. Once mounted on the animal, the entire VDAP unit is neutrally buoyant in water and weighs about 2 kg in air (about 1% of the mass of a juvenile and 0.7% of the mass of an adult female SSL). The recording instruments will augment hydrodynamic drag, but the cross sectional area of the largest instrument will be less than 2% of the maximum cross sectional area of the animal, so drag augmentation will not be large. Based on experience with Weddell seals, the VDAP system does not appear to diminish foraging and prey capture (Davis et al. 1999). The release system for the instruments can be remotely triggered to allow the devices to fall off. The low-profile, remote release baseplate eventually falls off when the sea lion molts in the fall. The intentional increase of drag or altered buoyancy via blocks is likely to alter dive and foraging behavior by a measurable amount. Similar manipulations have been performed on Antarctic fur seals (Boyd et al. 1997) and northern elephant seals (Webb et al. 1998). Fur seals that had their drag increased by a similar amount (approximately 10%) made longer foraging trips but were able to gain the same amount of mass as unmanipulated fur seals. Elephant seals whose buoyancy was increased or decreased by up to 100% displayed differences in ascent and descent rates but not in dive duration, trip duration, or mass change. Therefore, although we expect to gain insight into how Steller sea lions

cope with changes in buoyancy (due e.g., to changes in body condition or instrumentation) or work load (increased costs due to changes in fish distribution or instrument attachment), we do not anticipate that these changes will adversely impact individuals in a significant way. Because the manipulations will last no more than four weeks, any effect will be temporary and unlikely to compromise the long-term health of Steller sea lions.

Bioelectric impedance analysis – There is a small risk of infection associated with the insertion of the needles.

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

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

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

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

Dry holding* – There are no adverse effects anticipated with these procedures; however, animals will be sheltered from inclement environmental conditions when necessary to prevent overheating or freezing.

Fasting* – Studies of juvenile Steller sea lions have shown no overt detrimental effect of temporary fasting of up to two weeks (Mellish and Horning, in review).

Fecal enema – Insertion of a tube into the rectum can result in perforation, which can lead to peritonitis that may result in death.

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

Flipper tagging - These types of tags are best considered semipermanent markers as they can and do pull out because sea lions use their foreflippers in both aquatic and terrestrial locomotion. When the tag is affixed there is the potential for infection at the wound site, particularly because the environment on the rookery is not aseptic and because the activity of the animal may prolong or prevent healing by producing repetitive stress on the wound. There is also the potential for infection when a tag pulls out of the flipper. In moving about on a rookery or haulout, or swimming, there is the potential for a tag to be torn out of the flipper by abrasion on the substrate or by hydrodynamic pressure. There is

no quantitative information on the rate of infection caused by flipper tagging Steller sea lions.

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

Hot-brand – Brands are applied while the animal is under anesthesia in order to minimize pain. There is the potential for infection at the wound site, and the activity of the animal may prolong or prevent healing by producing repetitive stress on the wound. However, individuals typically appear to respond well to the procedure and show no clinical signs of infection or inflammation within weeks (Mellish et al. in press). There are two different branding techniques that provide permanent, uniquely identifiable, large marks: freeze-branding and hot branding. Freeze branding can be used to produce two different types of marks. Short contact by the branding iron (10-20 sec. per character) destroys pigment-producing cells and results in an unpigmented brand. Prolonged contact (> 45 sec. per character) destroys both hair and the pigment-producing cells for a bald brand. Hot branding involves brief (2-4 sec. per character) application of a red-hot iron to kill both hair follicles and pigment producing cells for a permanently bald brand.

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

The limited literature on hot versus freeze-branding suggests that both techniques cause discomfort and stress in unanesthetized animals, and neither technique is clearly better than the other from an ethical point of view. Studies on cattle have shown that both procedures stress the subject (Lay et al. 1992 a, b, c; Schwartzkopf-Genswein et al. 1997 a, b). Freeze branding produced a protracted chronic stress, partly due to the prolonged restraint of the animal and partly due to the slow thawing of the wound. A 3-digit bald freeze brand required 3 minutes or more for application of the brand, compared to 12 seconds for a comparable hot brand (Lay et al. 1992). Schwartzkopf-Genswein et al. (1997a) found that while neither technique affected weight gain after branding, freeze-branded cattle were more difficult to handle in the 10 days after branding, suggesting that cattle experienced a lingering pain from freeze-branding. Both techniques caused a rise in blood cortisol concentrations immediately after branding, with the hot branded cattle having the highest cortisol between 20 and 40 minutes after branding, but neither technique affected post-branding pain sensitivity compared with shams (Schwartzkopf-Genswein et al. 1997b). Thus, hot branding produces a brief acute stress, and the animal recovers more quickly than with freeze branding. Preliminary results from a study of the effects of freeze and hot branding harbor seals (*Phoca vitulina*) indicated there were no differences in heart or respiration rates (W. Stobo, Bedford Inst. Ocean, pers. comm. 1993). Anesthesia should help to alleviate the short term discomfort and stress of hot

branding, leading us to conclude that freeze-branding is not a suitable alternative to hot-branding in sea lions.

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

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

Material collection from haulouts and rookeries: Collection of materials will result in disturbance of sea lions due to the presence of researchers.

Metabolic chamber – During 2003, Dr. Rea (CI) conducted this experiment on 23 wild-caught animals with no noticeable effects, such that no adverse effects are anticipated and no mitigation is required.

Milk sample collection – There is a small risk of infection associated with the needle injection of oxytocin. Manipulation of the teats can result in bruising of the mammary tissue.

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

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

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

Stomach lavage - There is the risk of introduction of liquid into the trachea, initiating aspiration pneumonia or death. There is also a risk of cross-contamination if equipment is not properly disinfected between animals.

Temporary captivity* – All contact with husbandry and research personnel will be limited and a remote feeding system will be utilized such that no adverse effects have been noted (Mellish et al. 2006) or are anticipated in the future.

Temporary mark (i.e., grease stick, hair dye, shaved spot) – There are no adverse effects anticipated with these procedures.

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

Tooth extraction – There is a small risk of infection associated with pulling a tooth. Goebel et al. (2005) examined the long and short-term effects of non-lethal tooth extraction on Antarctic fur seals, and the only short-term effect was a minor effect on maternal attendance (on-shore visit duration was slightly longer after tooth extraction). Tooth extraction had no effect on over winter survival, fecundity, mass gain or diving behavior (Goebel et al. 2005).

Ultrasound – No adverse reactions are anticipated to this procedure.

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

X-ray* – The primary discomfort associated with this procedure is immobilization, which is eliminated with the use of anesthesia.

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

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

2. Measures to minimize effects

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

Task 1

Attachment of scientific instruments mitigation - The acetone and volatile components of the neoprene cement can be absorbed into the skin, but our technique is to apply it only to the distal half of the fur strands and therefore contact with the skin should be minimal. We use a slow-setting, low exothermic epoxy, so there is little chance of trauma to the skin.

Bioelectric impedance analysis mitigation: Only sterile needles will be used to minimize the chances of infection.

Blood sample mitigation: The blood volume of Steller sea lions varies from approximately 90 to 120 ml per kg^{-1} body mass (Richmond 2004). The acceptable safe

veterinary standard for blood withdrawal is 10% of total blood volume (Murray 2000). Therefore, for all Steller sea lions the acceptable safe limit for blood withdrawal would be at least 9 ml kg^{-1}, but we will be limiting ourselves to only 1 ml kg^{-1} when drawing blood. Therefore, we do not expect any adverse effects from this amount of blood collection. To reduce the risk of infection, only clean, sterile disposable needles will be used to obtain blood samples and a new needle will be used for each blood collection.

Capture, restraint and anesthesia, mitigation:

To minimize the effects of handling, we will use veterinarians and experienced biologists to watch for signs of distress, and release animals showing such signs. In addition, any animal showing signs of distress while being handled will be released immediately and closely monitored. An emergency kit with equipment and supplies for responding to complications or emergencies will be readily available. There is a risk of accidental death during capture and anesthesia. In addition, some animals could die during disturbance of the rookery, capture and handling, and infection from blood and tissue sampling. We will minimize disturbance to the rookery by working around the margins and avoid going into dense aggregations of animals. The risk of infection will be reduced through the disinfection of sampling sites and the use of sterile/aseptic equipment and sampling techniques. These procedures will only be performed by/under the direct supervision of qualified and experienced personnel. The capture noose will be fitted with a stop mechanism that prevents the noose from tightening too much so that strangulation or any other neck/trachea injury will be prevented.

To avoid respiratory distress, ischemia (restricted blood flow), or nerve damage, animals will be properly positioned, i.e. ventrally recumbent, during anesthesia. It is important to avoid prolonged breath holding during gas anesthesia as this can result in cardiac hypoxia (lack of oxygen to the heart muscle): therefore, respiration and blood oxygen saturation will be monitored and oxygen administered as needed.

Chemical immobilization mitigation: Chemical immobilization of pinnipeds can be problematic because of their cardio-respiratory adaptations for breath-hold diving. We will be employing the most prudent techniques and mitigation procedures for chemical immobilization as recommended in the recent "Environmental Assessment on the Effects of NMFS Permitted Scientific Research Activities on Threatened and Endangered Steller Sea Lions" (NMFS 2002).

Heath et al. (1996) have reported on 51 adult female Steller sea lions that were immobilized with Telazol between 1992 and 1994. Of these 51, there were five mortalities (9.8%). Only two of the mortalities were due to Telazol complications. Two sea lions drowned in pools of water on the rookery, and one sea lion died because of a malfunction of the gas anesthesia machine. With improved methods, it has been possible to reduce the risk of mortality associated with chemical immobilization. Between 1995 and 2006, two of us (Calkins and Andrews) have been involved with an additional 43 Telazol immobilization attempts of free-ranging Steller sea lions throughout the world, and we encountered only one mortality (2.3%; R.B. Heath and R.D. Andrews, unpublished data). A significant improvement in method was the addition of atropine sulfate to Telazol in the dart. Atropine is a muscarinic-receptor antagonist, which blocks the parasympathetic stimulus to reduce heart rate. This bradycardia is a key element of

the cardiovascular dive response that is sometimes mimicked during Telazol anesthesia. Atropine, therefore, reduces the cardio-respiratory complications of Telazol. Improvements were also made to the gas anesthesia machine to prevent the type of malfunction that occurred in the earlier design.

The mean induction time for Telazol anesthesia in adult female Steller sea lions is approximately 10 minutes. Therefore, one of the risks of chemical immobilization is that a sea lion can be spooked into the water before induction is complete. In order to minimize this risk, we will exclude sea lions that are close to the ocean shoreline or pools of standing water on the rookery. The darter and observer will be dressed in camouflaging clothes and move slowly and carefully to a position as close to the subject as possible without disturbing it or other sea lions, allowing the shot to be taken from as short a distance as possible. Darting from a short distance will permit lower impact velocities, thus reducing the startle effect of darting. Close proximity to the target sea lion will also facilitate more accurate estimates of mass and quicker access to the sea lion once induction has occurred. Quick access will reduce complications associated with compromised breathing when a sea lion becomes immobilized and adopts a posture that inhibits normal breathing patterns.

Estimation of body mass to calculate drug dosage can be done with an accuracy of ± 20 kg by an experienced biologist (Loughlin and Spraker 1989; Heath et al. 1996). We will however, attempt to tremendously improve this accuracy by using photogrammetry to measure body length of target sea lions. From a body length/body mass regression, we will be able to predict body mass much more accurately than was previously possible, reducing the possibility that target sea lions will be over or under-dosed.

As a precaution, we will have a skiff standing by with the appropriate nets and equipment for pulling a sea lion out of the water. If a darted sea lion does enter the water and then becomes immobilized, we will attempt to restrain it and bring it aboard the boat if it can be done safely.

Another concern when chemically immobilizing adult female sea lions is the effect of Telazol on the fetus or pup. Telazol has been shown to cross the placental barrier and therefore use of Telazol for Cesarean section in dogs and cats is contraindicated (Telazol drug information sheet; CI 5129-1; Fort Dodge Animal Health, Fort Dodge, IA). Telazol, however, causes less respiratory depression in the fetus than other commonly used injectable anesthetics, and therefore it is commonly used for Cesarean sections in monkeys and cats (M. LaRosh, D.V.M.; Fort Dodge Animal Health Veterinarian, pers. comm.). There have been no studies on the teratogenicity of Telazol. Although during the months of June–August, the adult female Steller sea lions that we target might be pregnant, the embryo will be at the arrested blastocyst stage, so any effects of Telazol will be negligible. The two components of Telazol, tiletamine and zolazopam are both lipophilic compounds with moderate half-lives (2-4 hours), so it might be possible for these compounds to be excreted in breast milk. There have been no studies of excretion of Telazol into milk, but other cyclohexamines and benzodiazapines have been examined. In human infants that nursed on mothers shortly after the mothers received high doses of diazepam, a benzodiazepine similar to zolazopam, the plasma concentration in the infant was typically less than 10% of the mother's concentration, which is well below the level that could produce anesthetic symptoms or other complications (Hale 1999; Lee and Rubin 1993). Semple et al. (2000) reported that the tissue residues of telazol in polar

bears were down to trace levels within 24 hours post-immobilization, and even at 12 hours the highest concentrations were still well below the level that could produce an anesthetic effect. It seems unlikely, therefore, that Steller sea lions could excrete enough Telazol in their milk to have an adverse effect on their pups. Based on the pharmokinetics of isoflurane, the amount of isoflurane that would be expected to be excreted in milk would be negligible (Lee and Rubin 1993). Furthermore, we have never heard any reports of anesthetic symptoms or other complications in the pups of immobilized Steller sea lions despite close visual observations of many of the immobilized females and their pups at Lowrie Island in Southeast Alaska.

The pups of lactating females that are targeted for capture will be identified before attempting to immobilize the female. In the event that the female dies or is seriously injured as a result of the activities, the orphaned pup shall be humanely provided for in consultation with the National Marine Fisheries Service Regional Office and ASLC rehabilitation and veterinary staff (i.e. salvaged [placed in a Stranding facility for rehabilitation and eventual release], or if salvage is not possible, euthanized).

If a sea lion does die as a result of these research activities, subsequent research will halt until qualified personnel can review the incident. In that event, all procedures will be evaluated and the research team (including a veterinarian) will attempt to identify the cause of the accidental mortality and to adjust procedures so as to prevent a reoccurrence. This will be a thorough review and will take as much time as required. We will not initiate another immobilization attempt unless it is deemed safe and prudent to continue. A report will be prepared for a mortality incident and it will be submitted to all relevant parties (e.g. ASLC IACUC, NMFS OPR and Regional Office.)

Deuterium oxide dilution mitigation: The primary discomfort associated with this procedure is blood collection. A maximum of three needle insertions to initially locate a vein and subsequently obtain the required blood volume will be made at any one site at any one session to reduce muscle injury and bruising and to reduce risk of introducing infection. 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.

Fecal enema mitigation: When performed by a qualified, experienced person using a blunt ended tube and commonly accepted standards of good practice, the risk of perforation is negligible. As animals will be immobilized for this procedure, the risks will be very minimal. The tubes will be washed and disinfected between use on different sea lions.

Flipper tagging mitigation: Care will be taken to avoid placing the tag so low as to have the animal walking on it or so high as to have it irritating the animal's flank area. To reduce the risk of infection, the area will be thoroughly disinfected with ethyl alcohol or betadine prior to applying the tag. In addition, the tags will be thoroughly cleaned and disinfected immediately prior to application. This procedure will only be performed by/under the direct supervision of qualified and experienced personnel.

Hot brand mitigation: Only experienced personnel will conduct the branding operation. To minimize the effects, we will use experienced personnel to watch for signs of distress,

and release animals showing such signs. In addition, any animal showing signs of distress while being handled will be released immediately and closely monitored. An emergency kit with equipment and supplies for responding to complications or emergencies will be readily available. Brands are applied while the animal is under anesthesia in order to minimize pain. Only clean brands heated to the appropriate temperature will be used by experienced personnel.

Material collection from haulouts and rookeries; mitigation: Material collection will be done by trained personnel who will work to minimize the overall disturbance by dressing in camouflaged clothing, moving with stealth when near sea lions, and, once sea lions are disturbed, completing the collection process as quickly as possible so as to minimize the time that sea lions are displaced. Collection using the remotely-controlled vehicle will be restricted by the following condition: caution must be exercised when approaching mother-pup pairs, and efforts to approach a particular animal or mother/pup pair must be terminated if there is any evidence that the activities may be life-threatening or interfering with the animals vital functions, or if the presence of the vehicle is causing obvious signs of distress.

Milk sample collection mitigation: Care will be taken to avoid trauma to the mammary gland and excessive suction will be avoided. This procedure will only be performed by/under the direct supervision of qualified and experienced personnel.

Skin/mucosal swab mitigation: To reduce the risk of infection, only clean, sterile disposable swabs will be used. This procedure will only be performed by/under the direct supervision of qualified and experienced personnel.

Skin sample collection mitigation: To reduce the risk of infection, the area will be thoroughly disinfected with isopropyl alcohol or betadine prior to applying the punch tool and inserting the flipper tag. This procedure will only be performed by/under the direct supervision of qualified and experienced personnel.

Stomach lavage mitigation: Only experienced, qualified personnel (veterinarians, biologists) who know how to properly pass a stomach tube to avoid introduction of liquid into the trachea will attempt this procedure. Tubes will be properly washed, disinfected, rinsed, and shaken or spun dry between animals.

Temporary marking mitigation: Marking of restrained sea lions with hair bleach is very safe and is a method that has been used on many Steller sea lion pups (e.g. Adams 2000). There is a small risk when using remote projection that the marking fluid could hit a sea lion in the eye. This risk can be significantly minimized by using this method from a close distance and by only projecting marking balls when wind speed is low. This procedure will only be performed by qualified and experienced personnel.

Tissue collection mitigation: Procedures will be conducted with sedation or anesthesia. To reduce the risk of infection, only clean, sterile disposable scalpel blades or biopsy punch tool will be used to obtain biopsy samples and a new scalpel blade or biopsy punch

tool will be used for each biopsy. Scalpel blades and biopsy punch tools will not be re-used on individual animals or between animals. The area to be sampled will be thoroughly disinfected with 20% isopropyl alcohol or dilute povidone iodine/saline solution prior to insertion of the cutting instrument. Only personnel with sufficient experience in the technique will be allowed to perform this procedure.

Tooth extraction mitigation: Only qualified veterinarians or other personnel with sufficient experience in the technique will be allowed to perform this procedure. The animal will be anesthetized and respiration will be closely monitored.

Task 2

Attachment of scientific instruments – There is no mitigation required for this activity.

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

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

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

obtain blood samples and a new needle will be used for each blood collection.

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

Dry holding* – Animals will be sheltered from inclement environmental conditions when necessary to prevent overheating or freezing.

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

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

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

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

Metabolic chamber – There is no mitigation required for this activity.

Morphometrics – There is no mitigation required for this activity.

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

Temporary captivity* – All contact with husbandry and research personnel will be limited and a remote feeding system will be utilized such that no adverse effects have been noted (Mellish et al. 2006) or are anticipated in the future.

Temporary mark (i.e., grease stick, hair dye, shaved spot) – There is no mitigation required for this activity.

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

Ultrasound – There is no mitigation required for this activity.

Whisker extraction – There is no mitigation required for this activity.

Fasting* – Mass will be monitored up to daily via voluntary placement on a platform scale. Any individual which exceeds 3% mass loss per day for more than three days will be removed from the study.

X-ray* – The primary discomfort associated with this procedure is immobilization, which is eliminated with the use of anesthesia. There is no mitigation required for this activity.**3. Monitoring effects of activities**

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

Animals are monitored post-procedure until they have completely recovered from the anesthesia or handling procedure. All free-range animals are maintained in a cool, dry area until complete recovery from anesthesia prior to release. Transient juveniles are maintained in a dry holding area until complete anesthetic recovery is achieved prior to pool access. Both free-range pups and juveniles and temporarily captive transient juveniles are hot-branded and satellite tagged whenever possible to facilitate long-term monitoring.

The remote video system provides a unique and excellent opportunity for continuous behavioral monitoring of animals following procedures in the wild and for released animals.

4. Alternatives

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

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

E. Resources Needed to Accomplish Objectives

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

TASK 2. The research included in Task 2 of this application is funded via various sources including Federal Appropriations to ASLC (ASLC #R2932), Steller Sea Lion Research Initiative (NOAA #NA17FX1429, #NA17FX1430) and the National Science Foundation (Major Research Instrumentation #480431).

F. Publication of Results

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

Research results will be published and made available in the appropriate refereed scientific journals at the discretion of the investigators involved with the respective research programs to be implemented.

V. National Environmental Policy Act (NEPA) Considerations

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

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

2. Does your activity involve the collection, handling, or transport of potentially infectious agents or pathogens, and/or does your activity involve the use or transport of hazardous substances?

Yes. The collection and transport of biological samples will only occur with proper sample identification and personal protective equipment (e.g., latex gloves, close-toed shoes).

3. If any of your activities occur in or near unique geographic areas, would any aspect of your activities impact the physical environment?

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

4. Do you know if your work could affect entities listed in or eligible for listing in the National Register of Historic Places, or cause loss or destruction of scientific, cultural or historic resources?

No such entities or resources are anticipated to be affected by this work.

5. Would your proposed activities include actions that might involve the transport of any material, biological or otherwise, from one area to another?

No, all transport of animals would occur within the indigenous area.

VI. Previous and Other Permits

A. Previous permits:

National Marine Fisheries Service, Office of Protected Resources (#881-1443, 881-1668, 881-1724)

B. Other permits:

US Department of Agriculture, Animal and Plant Health Inspection Service (#96-R-0005)

US Department of the Interior, Fish and Wildlife Service (#01-015)

US Department of the Interior, Fish and Wildlife Service, Alaska Maritime National Wildlife Refuge (Special Use Permit No. 74500 – 03-045)

US Department of Homeland Security, US Coast Guard (DTCG-Z71117-02-RP-025L)

US Department of Defense, Army Corps of Engineers Nationwide Permit No. 5

State of Alaska, Department of Natural Resources, Division of Mining, Land and Water (Land Use Permit No. LAS 25187)

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VIII. Certification and Signature

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

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

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

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

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

Signature of applicant _____

Date _____

Typed or Printed Name of Applicant: Tylan Schrock_____

Title of Applicant Executive Director, Alaska Sealife Center_____

Appendix A. Transient Juvenile Project Health Screening Protocol.

All animals undergo a health screen protocol prior to admission to the ASLC South Beach facility, as well as prior to release. The monitoring of the physical condition, body condition, general health and evidence of disease in juvenile Steller sea lions while being held temporarily in captivity is imperative to (1) ensure these sea lions remained clinically healthy throughout their stay in temporary captivity and (2) to ensure that these animals do not place the wild population of Gulf of Alaska Steller sea lions, from which they came, at risk following their release. In order to accomplish these goals, clinical samples must be collected from both juveniles entering temporary captivity and those that are immediately released following capture to be tested and compared to ascertain baseline values for each, as well as sequentially from juveniles maintained in captivity in order to be able to identify and monitor changes that may occur over time. In particular, an important aspect of this sampling regime is the screening for normal and pathogenic bacteria in swab samples and testing for changes in antibiotic resistance over time. There is evidence of increasing antibiotic resistant bacteria present in wildlife (O'Rourke, 2003), and it is important to ascertain that this program does not contribute to this problem as a result of bringing these animals temporarily into a captive facility. Additionally, it is necessary to determine that juvenile Steller sea lions are not exposed to novel diseases while in temporary captivity and that any evidence of exposure to disease is similar at capture and release, as well as is similar to evidence of exposure in their free-ranging cohorts.

Sample collection includes: blood, swabs, feces, mucus, blubber, skin and hair. All animals are sampled at capture (both juveniles entering temporary captivity and those that are immediately released), and juveniles maintained in captivity are sampled routinely until release. General health monitoring includes examination of whole blood for hematologic analysis (WBC, Differential count, RBC, HCT, HgB, MCV, MCH, MCHC, Platelets) and harvesting of serum for serum biochemistry analysis (Glucose, BUN**,** Creatinine, Potassium Calcium, Albumin, Globulin, Total protein, Total bilirubin, Alkaline phosphatase, ALT, Amylase, Cholesterol). Serologic analysis is performed to test for evidence of exposure to common marine mammal and terrestrial mammal pathogens. Sera are submitted for testing upon capture and prior to release (within two weeks) for antibodies to bacterial pathogens: *Brucella spp.*, *Leptospira spp.* (serovars *bratislava, canicola, grippophytosa, hardjo, icterohaemorrhagica, pomona)*; viruses: Morbilliviruses (canine distemper, phocine distem per, dolphin morbillivirus, porpoise morbillivirus), Phocine herpesvirus-1, Calicivirus serotype 1(San Miguel sea lion virus); and protozoa: *Toxoplasma gondii, Sarcocystis neurona*.

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

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

To date a total of 86 sea lions have been sampled as a result of this program (31 that were held temporarily in the facility and 55 captured and released immediately). Results thus far show that Steller sea lions held in temporary captivity for up to three months remained healthy. Evidence of wild exposure to a herpesvirus and *Leptospirosis spp*. was found in a small number of animals, both those that entered captivity as well as in those that were captured and released. Antibiotic resistance was found to three antibiotics (Amoxicillin/clavulanic acid, Ampicillin, Cefazolin) in two *E. coli* isolates from one sea lion upon capture prior to entering captivity; however, upon release, all isolates were found to be susceptible to all antibiotics. No isolates from other animals tested showed any antibiotic resistance. To date, animals maintained temporarily in captivity have not shown any evidence of exposure to novel diseases or developed antibiotic resistance that could place free-ranging animals at risk for disease following their release. It is important to continue to monitor the health of these juveniles while in temporary captivity to ensure this is maintained.

The health screen typically includes, but is not limited to, the following procedures:

ENTRY

1. Physical inspection for broken bones, major wounds and/or inflammation
2. CBC (white blood cells, hematocrit, platelet counts)
3. Serum chemistry (albumin, blood urea nitrogen, calcium, total bilirubin, potassium, total protein, globulins)
4. Body condition via ultrasound

Samples are also collected at the entry point for the following non-immediate analyses:

1. Viral serology
2. Skin and mucosal swabs
3. Contaminants (blubber biopsy, hair)
4. Nutritional status (whisker, blubber biopsy, deuterium oxide)

INTERIM (up to weekly* during captivity period)

1. Physical inspection for broken bones, major wounds and/or inflammation
2. CBC (white blood cells, hematocrit, platelet counts)
3. Serum chemistry (albumin, blood urea nitrogen, calcium, total bilirubin, potassium, total protein, globulins)
4. Body condition via ultrasound

*Frequency of interim sampling will be dependent on the specific research requirements per cohort.

EXIT

At any point up to release the following procedures will be performed:

1. Physical inspection for broken bones, major wounds and/or inflammation
2. CBC (white blood cells, hematocrit, platelet counts)

3. Serum chemistry (albumin, blood urea nitrogen, calcium, total bilirubin, potassium, total protein, globulins)
4. Body condition via ultrasound
5. Viral serology
6. Skin and mucosal swabs
7. Nutritional status (whisker, blubber biopsy, deuterium oxide)

APPENDIX B
Alaska SeaLife Center
Carcass/Tissue Handling Protocol

To avoid any contamination, the following procedures must be followed when obtaining or receiving a DOA animal or associated tissue. This includes collection, research and stranded animals found dead within the facility as well as stranded DOA animals brought back to the Alaska SeaLife Center.

FOR YOUR PERSONAL PROTECTION DO NOT touch any animal carcasses or tissue without first putting on latex or vinyl gloves.

1. Survey the scene before touching the carcass. This is very important in the diagnostic/forensic process to determine cause of death. Location of body, position of body, signs of other animals in the area, distance from water and power lines in the area are all important details. Be sure to take a camera and take at least ten pictures total of the carcass, surrounding area and anything that may look suspicious.
2. Describe the physical condition in which the body is found (fresh, scavenged, decomposed).
3. Collect a history if possible. Question bystanders who may provide details regarding the length of time the carcass has been there, or any information on the animal prior to death (i.e. was the animal seen alive?).
4. Record carcass information collected. Include narrative, descriptions, times, dates, and names. Submit with other paperwork. Sign your name to any information you record.
5. Put all contents in a minimum of two layers of plastic bags. If there is an excessive amount of fluid, multiple bags or a tub may be required. Place the carcass into the first bag and close with a twist tie or cord. Place that into another bag avoiding any contamination of the outside of the 2nd bag. Do not touch this outside bag with your contaminated gloves. Put contaminated gloves into 2nd bag and seal securely. Two persons may be needed. This is very important to avoid any contamination in the transport vehicle, the Alaska SeaLife Center or any person handling the carcass.
6. If the carcass is already frozen, put the doubled bagged material into the necropsy freezer following the procedures listed in the necropsy log.
7. If the carcass is not frozen, put the doubled bagged material in a plastic tub in the necropsy room refrigerator or into a cooler covered with ice. Do not put ice directly into contact with the carcass.
8. Return all records to either the stranding office or security.
9. Contact either the Senior Veterinarian, Associate Veterinarian, Rehab Coordinator, Lab Manager, Senior Veterinary Technician, Stranding Office (x6395), Stranding Hotline (1-888-774-7325 (SEAL)), or Security (x3642).

APPENDIX C
Alaska SeaLife Center
Euthanasia Protocol

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

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

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

Pentobarbitol products may be used for euthanasia of most amphibians and fish at a rate of 60 mg/kg of body weight by intravenous, intra abdominal or intra pleuroperitoneal injection. Tricaine methanesulfonate (MS222) may be used at a minimum of 300 ppm in water to sedate these species prior to lethal injection or to use of physical methods of ensuring death (as described below).

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

Stunning, followed immediately by exsanguination, pithing or decapitation is an acceptable alternative for fish, amphibians and smaller bird and mammal species provided that the blow or electric current produces immediate unconsciousness of sufficient duration to ensure that secondary procedures can be completed before the animal regains sensation.

In selected cases, an accurately placed gunshot may be considered acceptable for very large or dangerous animals. This method should only be used by highly skilled personnel using a firearm appropriate for the situation. The firearm should be aimed so that the projectile enters the brain, causing immediate unconsciousness.

All research protocols shall specify a planned method of euthanasia and carcass disposition for use in case of emergencies even if death as an end point is not part of the proposal. The IACUC Assurance of Animal Care Form for mammals and birds should indicate injection of pentobarbitol euthanasia solution as described above and refer to this protocol as a default plan. Alternative methods may be considered but if the desired method involves pain or distress to the animals which is not relieved by appropriate anesthetic, analgesic or tranquilizing drugs, the proposal must include scientific justification and an explanation of the procedures and the reasons why such drugs were not used.

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

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

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

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

1. Collection animals

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

2. Research animals

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

3. Rehabilitation animals

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

Table 1. Proposed activities under Task 1 for Steller sea lions between 01 July 2007 and 30 June 2012.

Species	Life Stage	Sex	Expected Annual Take import/ export	# takes per individual	Take Action	Transport	Location	Dates/ Time period
Eumetopias jubatus	pup 5 d–2 mo	m/f	200	1	-capture/restraint -morphometrics -digital imaging -ultrasound -blood/skin samples -temporary mark (ie, grease stick, dye) -hot-brand or flipper tag	n/a	Sugarloaf Is., Outer (Pye) Is., Chiswell Is., Seal Rocks (Prince William Sound), Fish Is., Cape St. Elias.	June – July (inclusive)
Eumetopias jubatus	all ages	m/f	10000	U	incidental disturbance during capture/sampling, scat and carcass collection or remote-video monitoring and other observational activities	n/a	West of 144 deg W.	year-round
Eumetopias jubatus	all	m/f	Unlimited	1	collection of expelled placentas, aborted fetuses, carcasses and organs from dead sea lions	via vessel , land and/or air	West of 144 deg W.	year-round
Eumetopias jubatus	all	m/f	5	1	Incidental mortality	n/a	West of 144 deg W.	year-round
Eumetopias jubatus	Pup 5 d – 2 mo	m/f	40	5	- Capture/restraint (by hand, hoop net, lasso, floating trap, or isoflurane) - Digital imaging - Morphometrics - Stomach lavage - Fecal collection by enema	n/a	West of 144 deg W.	June - July

Species	Life Stage	Sex	Expected Annual Take import/ export	# takes per individual	Take Action	Transport	Location	Dates/ Time period
					- Isotopic water dilution - Bioelectric impedance analysis - Ultrasound - Blood samples - Dermal / mucosal swabs - Skin samples - Blubber/muscle biopsy collection - Whisker extraction - Scientific Instrument Attachment - Temporary mark - Hot-brand or Flipper Tagging			
Eumetopias jubatus	Pup 2 mo to 1 yr	m/f	40	3	- Capture/restraint (by hand, hoop net, lasso, floating trap, or isoflurane) - Digital imaging - Morphometrics - Stomach lavage - Fecal collection by enema - Isotopic water dilution - Bioelectric impedance analysis - Ultrasound - Blood samples - Dermal / mucosal swabs	n/a	West of 144 deg W.	Aug - May

Species	Life Stage	Sex	Expected Annual Take import/ export	# takes per individual	Take Action	Transport	Location	Dates/ Time period
					- Skin samples - Blubber/muscle biopsy collection - Tooth Extraction (only 1 per lifetime) - Whisker extraction - Scientific Instrument Attachment - Temporary mark - Hot-brand or Flipper Tagging (only 1 per lifetime)			
Eumetopias jubatus	> 1 yr to 4 yrs	m/f	40	3	- Capture/restraint (by hand, hoop net, lasso, floating trap, or isoflurane) - Digital imaging - Morphometrics - Stomach lavage - Fecal collection by enema - Isotopic water dilution - Bioelectric impedance analysis - Ultrasound - Blood samples - Dermal / mucosal swabs - Skin samples - Blubber/muscle biopsy collection - Tooth Extraction (only 1 tooth per	n/a	West of 144 deg W.	year-round

Species	Life Stage	Sex	Expected Annual Take import/ export	# takes per individual	Take Action	Transport	Location	Dates/ Time period
					lifetime) - Whisker extraction - Scientific Instrument Attachment - Temporary mark - Hot-brand or Flipper Tagging (only 1 brand or tag event per lifetime)			
Eumetopias jubatus	Adult	F	60	1	- Capture/restraint (by hand, hoop net, lasso, floating trap, chemical immobilization or isoflurane) - Digital imaging - Morphometrics - Stomach lavage - Fecal collection by enema - Isotopic water dilution - Bioelectric impedance analysis - Ultrasound - Blood samples - Dermal / mucosal swabs - Skin samples - Blubber/muscle biopsy collection - Tooth Extraction - Whisker extraction - Scientific Instrument	n/a	West of 144 deg W.	Aug - May

Species	Life Stage	Sex	Expected Annual Take import/ export	# takes per individual	Take Action	Transport	Location	Dates/ Time period
					Attachment - Temporary mark - Hot-brand - Milk collection			
Eumetopias jubatus	Adult	F	40	3	- Capture/restraint (by hand, hoop net, lasso, floating trap, chemical immobilization or isoflurane) - Digital imaging - Morphometrics - Stomach lavage - Fecal collection by enema - Isotopic water dilution - Bioelectric impedance analysis - Ultrasound - Blood samples - Dermal / mucosal swabs - Skin samples - Blubber biopsy collection - Tooth Extraction (only 1 tooth per lifetime) - Whisker extraction - Scientific Instrument Attachment - Temporary mark - Hot-brand or Flipper	n/a	West of 144 deg W.	June - July

Species	Life Stage	Sex	Expected Annual Take import/ export	# takes per individual	Take Action	Transport	Location	Dates/ Time period
					Tagging (only 1 brand or tag event per lifetime) - Milk collection			
Eumetopias jubatus	Adult	F	40	3	- Capture/restraint (by hand, hoop net, lasso, floating trap, chemical immobilization or isoflurane) - Digital imaging - Morphometrics - Stomach lavage - Fecal collection by enema - Isotopic water dilution - Bioelectric impedance analysis - Ultrasound - Blood samples - Dermal / mucosal swabs - Skin samples - Blubber/muscle biopsy collection - Tooth Extraction (only 1 tooth per lifetime) - Whisker extraction - Scientific Instrument Attachment - Temporary mark - Hot-brand or Flipper	n/a	West of 144 deg W.	Aug - May

Species	Life Stage	Sex	Expected Annual Take import/ export	# takes per individual	Take Action	Transport	Location	Dates/ Time period
					Tagging (only 1 brand or tag event per lifetime) - Milk collection			

Table 2. Proposed activities under Task 2 to be conducted between 01 July 2007 and 30 June 2012.

Species	**Life Stage**	**Sex**	**Expected Annual Take import/ export**	**# takes per individual**	**Take Action**	**Transport**	**Location**	**Dates/ Time period**
Eumetopias jubatus	pup 6-11mo	m/f	60	1	-capture/restraint -morphometrics -digital imaging -ultrasound -blood/tissue -deuterium oxide -opportunistic fecal/urine collection -hair sample -skin/mucosal swab -temporary mark (ie, grease stick, dye) -hot-brand -attachment of scientific instruments	n/a	Resurrection Bay Prince William Sound Kodiak Island Aleutian Islands	year-round
Eumetopias jubatus	juvenile 1-4yr	m/f	90* see below for transient juvenile subset	1	-capture/restraint -morphometrics -digital imaging -ultrasound -blood/tissue -deuterium oxide -opportunistic fecal/urine collection -hair sample -skin/mucosal swab -temporary mark (ie, grease stick, dye) -hot-brand -metabolic chamber -attachment of scientific instruments	n/a	Resurrection Bay Prince William Sound Kodiak Island Aleutian Islands	year-round

Eumetopias jubatus	all ages	m/f	4000	U	incidental harassment	n/a	Resurrection Bay Prince William Sound Kodiak Island Aleutian Islands	year-round
Eumetopias jubatus	all ages	m/f	2	1	unintentional mortality	n/a	Resurrection Bay Prince William Sound Kodiak Island Aleutian Islands	year-round
Eumetopias jubatus	all ages	m/f	unlimited	1	carcass collection	n/a	Resurrection Bay Prince William Sound Kodiak Island Aleutian Islands	year-round

Table 3. Proposed sub-activities for transient juvenile Steller sea lions between 01 July 2007 and 30 June 2012. Please note that these are a subset of the individuals taken under **Table 2**.

Species	**Life Stage**	**Sex**	**Expected Annual Take import/ export**	**# takes per individual**	**Take Action**	**Transport**	**Location**	**Dates/ Time period**
Eumetopias jubatus	juvenile 1-4yr	m/f	30	1	temporary captivity (≤ 3 months)	via vessel and/or land transport	South Beach Facility, ASLC	year-round
Eumetopias jubatus	juvenile 1-4yr	m/f	30	U	-digital imaging -opportunistic fecal and urine collection	n/a	South Beach Facility, ASLC	year-round
Eumetopias jubatus	juvenile 1-4yr	m/f	30	≤12	-capture/restraint -morphometrics -digital imaging -ultrasound -blood sample -skin/mucosal swab -temporary mark (ie, grease stick, dye)	n/a	South Beach Facility, ASLC	year-round
Eumetopias jubatus	juvenile 1-4yr	m/f	30	≤4	-tissue sample -deuterium oxide	n/a	South Beach Facility, ASLC	year-round
Eumetopias jubatus	juvenile 1-4yr	m/f	30	≤2	-x ray -whisker extraction -hair sample -metabolic chamber	n/a	South Beach Facility, ASLC	year-round
Eumetopias jubatus	juvenile 1-4yr	m/f	30	1	-hot-brand -attachment of scientific instruments - LHX implantation	n/a	South Beach Facility, ASLC	year-round
Eumetopias jubatus	juvenile 1-4yr	m/f	12	≤2	temporary dry holding (≤48hr)	n/a	South Beach Facility, ASLC	year-round
Eumetopias jubatus	juvenile 1-4yr	m/f	12	≤2	-short-term fasting (≤ 10d)	n/a	South Beach Facility, ASLC	year-round