Alaska SeaLife Center
windows to the sea

# I. AMENDMENT REQUEST FOR PERMIT 881-1745-00 FOR SCIENTIFIC RESEARCH TO ENHANCE THE SURVIVAL AND RECOVERY OF A STOCK UNDER THE ENDANGERED SPECIES ACT AND THE MARINE MAMMAL PROTECTION ACT

## II. DATE OF APPLICATION December 1, 2006

## III. APPLICANT AND PERSONNEL

### A. Applicant

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**Co-Investigators**

Russ Andrews, Ph.D.
Don Calkins
~~**Dennis Christen**~~
Lisa Hartman
**Justin Jenniges**
**Brett Long**
**Kendall Mashburn**
Jo-Ann Mellish, Ph.D.

**Cooperating Institutions**

**Mystic Aquarium**
**Oregon Coast Aquarium**
**Vancouver Aquarium**

**B. Qualifications and Experience**

1. Shannon Atkinson, Ph.D., Science Director, Alaska SeaLife Center- updated CV is attached.
2. Russ Andrews, Ph.D., Scientist, Alaska SeaLife Center- CV on file with the Office of Protected Resources.
3. Don Calkins, Steller Sea Lion Program Manager, Alaska SeaLife Center- CV on file with the Office of Protected Resources.
4. ~~**Dennis Christen**~~ **no longer works for ASLC. Please remove him from the permit.**
5. Lisa Hartman- CV on file with the Office of Protected Resources.
6. 
7. **Justin Jenniges, Research Field Coordinator, Alaska SeaLife Center, has over 4 years experience capturing and sampling Steller and California sea lions, as well as Pacific harbor and northern fur seals. His abbreviated CV is included.**
8. **Brett Long, Mammal Curator, Alaska SeaLife Center, has 15 years experience in the husbandry and training of many species of breeding and nonbreeding captive marine mammals. They include, but are not limited to *Tursiops truncatus*, *Zalophus californianus*, *Eumetopias jubatus*, *Phoca vitulina*, *Mirounga angustirostris*, and *Callorhinus ursinus*. Since 1997, he has also worked on several research projects studying the foraging ecology of California sea lions and Pacific harbor seals along the California coastline and Channel Islands. His CV is included.**
9. **Kendall Mashburn, Science Program Coordinator, Alaska SeaLife Center, has over 16 years experience working in the field of endocrine physiology and to date, has investigated both stress and reproduction in over 65 species of mammals, fish, birds and invertebrates. She spent 10 years with the Conservation and Research Center at the Smithsonian Institution researching reproductive and stress physiology in endangered species and her recent work with the ASLC includes season, gender and age specific adrenal and reproductive endocrine physiology in the Steller sea lion. Her abbreviated CV is included.**
10. Jo-Ann Mellish, Ph.D.- CV on file with the Office of Protected Resources.

**C. Attending Veterinary Staff**

1. Pam Tuomi, DVM, Senior Veterinarian, Alaska SeaLife Center- CV on file with the Office of Protected Resources.
2. Carrie Goertz, DVM, Associate Veterinarian- CV on file with the Office of Protected Resources.
3. Millie Gray, Senior Veterinary Technician, Alaska SeaLife Center- CV on file with the Office of Protected Resources.

## IV. DESCRIPTION OF THE PROPOSED SCIENTIFIC RESEARCH

### A. Summary

**Physiology and immunology of gestation and lactation:**
We request to add a 5 year study to assess physical, metabolic, hormonal, and immunological changes related to breeding of captive Steller sea lions (*Eumetopias jubatus*) maintained by the Alaska SeaLife Center, in Seward Alaska (NOAA permit 881-1745). This study may require the transfer of additional animals from Mystic and/or Oregon Coast Aquarium, and/or import from Vancouver Aquarium. Offspring produced during this study will become permanent residents within the Alaska SeaLife Center (ASLC) research program, or they may be transferred to Mystic or Oregon Coast Aquarium, and/or exported to Vancouver Aquarium. Regardless of location, these permanently captive progeny will help scientists answer questions about their wild counterparts.

The current application requires several types of additional adult takes beyond those already permitted in 881-1745, including multiple copulations and parturitions, as well as collection of milk samples. This study also requires additional tests upon blood, urine, and fecal samples already allowed under current ASLC permits. In addition, we seek to modify collection dates and methodologies approved in 881-1745.

We request that any offspring (up to 4 live births) produced during this study be added to this permit. We also request takes for these offspring, including morphometric measurements (i.e. mass, length, girth), metabolic measurements, collection of urine and feces, blood draws, and various types of audio and visual recordings (e.g. audio, photographic, video, digital, thermal, radiographic). Offspring will be trained by Husbandry staff, to voluntarily submit to as many of these takes as possible.

### B. Introduction

**Description of the Marine Mammals to be Taken:**

**Species and Stocks:** We request authority to take Steller sea lions (*Eumetopias jubatus*) during 5 years of continuing research, through March 31, 2011. This request applies to captive eastern stock Steller sea lions imported in 1998 under NOAA permit 881-1443 that are maintained at Alaska SeaLife Center, any animals transferred from cooperating institutions under current and future memorandums of understanding, and any future progeny described in this application. Additional, non-target species will not be present or affected.

**Background:**

**Status of Affected Population and Stocks:** Following a well documented decline of more than 80% in the last 30 years, Steller sea lions were designated as "threatened" range-wide under the U.S. Endangered Species Act (ESA). Two distinct populations, a western and an eastern stock, were identified with the division at Cape Suckling, Alaska (144ºW). Additional declines resulted in an "endangered" up-listing of the western stock, while

eastern populations kept the "threatened" designation due to a slow (approx. 3% per year) population increase (Draft Steller sea lion Recovery Plan, 2006). Steller sea lions are listed as "depleted" under the Marine Mammal Protection Act (MMPA).

Current research suggests that fecundity of western stock females has decreased sufficiently that the stock has limited ability to recover (Holmes et al., *in review*). It is widely accepted that nutritional and reproductive requirements affect fecundity to some extent, and that positive and progressive studies utilizing captive animals are needed to better understand these requirements (research task 2.5.4 in the Draft Revised Recovery Plan). As such, the Alaska SeaLife Center proposes to establish a formal propagation program not for the purposes of reintroduction, but to better understand the reproductive physiology of Steller sea lions. The enhanced understanding of reproductive processes and requirements should enable us to identify critical stages in the physiology of Steller sea lions such that we can predict potential reproductive losses among free-ranging counterparts.

**Reproduction:** Despite several studies describing the reproductive biology of Steller sea lions, little data exists characterizing the physiology of the annual reproductive cycle for this species. Steller sea lions appear to have low reproductive success relative to other pinnipeds. Shortly after the breeding season, 100% of the adult females are believed to be pregnant, yet studies examining sacrificed free-ranging animals estimated only 58-63% of those females deliver pups (Pitcher and Calkins 1981; Pitcher et al. 1998). These reproductive failures include fetal resorption and spontaneous abortion, pseudo-pregnancy or failure to conceive, and interference during embryonic diapause or implantation. The mechanisms controlling reproductive success or failure are poorly understood, but clearly reproductive hormones play an important role. Research on captive Steller sea lions can help establish the relationship between these processes and define critical periods where pregnant individuals may be more susceptible to reproductive failure.

**Endocrinology:** Research task 1.3.2 of the Draft Steller sea lion Recovery Plan (NMFS 2006) identified the need to develop improved indices of health, body condition, and reproductive status utilizing chemical methods such as endocrinology. There are inherent challenges in monitoring endocrine changes in large, free-ranging marine animals. For example, serial blood samples needed for endocrine analysis of known individuals in the wild are, by nature, difficult or impossible to obtain, and associated handling stress could perhaps disrupt the reproductive events being studied. Alternate methods have been developed in a variety of free-ranging terrestrial species to gather endocrine data. Studies of fecal monitoring techniques have provided researchers with a readily accessible, non-invasive means of studying steroid endocrinology of large mammals (Schwarzenberger et al. 1996; Gardiner et al. 1999; Pickard 2001; Mashburn and Atkinson 2004, 2006; Petrauskas and Atkinson 2006).

Reproductive steroids are secreted into the bloodstream, metabolized by the liver, excreted in bile, and voided in feces. Steroid analysis has proven to be a useful tool in characterizing reproductive studies in large terrestrial mammals including African elephants, sable antelope, Hokkaido brown bears, elk and spotted hyenas (Wasser et al.

1996; Thompson et al. 1998; Ishikawa 2003; Cook et al. 2002; Dloniak et al. 2004). This method has been successfully applied to marine mammals including, Hawaiian monk seals, California sea lions, North Atlantic right whales, sea otters and Florida manatees (Pietraszek and Atkinson 1994; Rolland et al. 2005; Larson et al. 2003; Larkin et al. 2005; Greig et al. 2006).

Glucocorticoids have been found to interfere with many aspects of female reproduction including estrus behavior, ovulation and cycle length (Vighio and Liptrap 1990; Gee et al. 1991; Asa and Ginther 1982). Among mammals, reproductive success depends on a series of intricate events proceeding in a defined temporal sequence, such that interference during any phase of the reproductive cycle can be deleterious. This becomes especially critical in species, such as Steller sea lions, that breed once a year. In these animals, altered behaviors, lack of ovulation, underdeveloped oocytes, and late breeding can result in either lack of pregnancy or unsustainable pregnancies that result in embryonic resorption or spontaneous abortion. During gestation, overexposure of fetuses to glucocorticoids disturbs the fetal hypothalamic-pituitary-adrenal axis and may contribute to intrauterine growth retardation (Lesage et al. 2001) or embryonic resorption. Even male reproductive processes, such as spermatogenesis, have been shown to be affected by long-term exposure to glucocorticoids (Liptrap 1993). However, it still appears that some glucocorticoid secretion is essential to successful pregnancy and parturition (Jacobs et al. 1994; Sun et al. 1996).

Researchers at ASLC have developed methods for investigating endocrine levels and associated physiological function in Steller sea lions (Mashburn and Atkinson 2004, 2006; Petrauskas et al. 2005; Petrauskas and Atkinson 2006; Myers et al. 2006). These methods have been successfully employed in both laboratory and field settings and have set the stage for assessment of free ranging populations. The next important issue that needs to be addressed is the relationship between normal hormonal concentrations at the onset of pregnancy to those found post-parturition and during lactation.

Most fecal samples used in endocrine analysis have been collected during seasons when reproduction and maternal care are at their height and increased adrenal activity would be expected (Challis et al. 2000; Whittle, et al. 2001; Mashburn and Atkinson 2006). Extreme variation in seasonal corticosterone concentrations among males could be mirrored by a similar increase in late gestational or early lactational females (Mashburn and Atkinson 2006). Since fecal corticosterone appears to change radically with gender and season, it is critical to understand the potential contributions to reproductive success being made by elevated concentrations of glucocorticoids and the “cut-off” point at which these concentrations become detrimental. Thus, it is part of the proposed study to monitor the longitudinal fecal glucocorticoid concentrations associated with reproduction, pregnancy, parturition and lactation in a controlled setting in order to establish baseline adrenal physiology in Steller sea lions.

Research task 1.2.4 of the Draft Steller sea lion Recovery Plan (NMFS 2006) indicates the development of methods to determine reproductive rates. Progesterone, the hormone most closely associated with pregnancy detection and support, typically increases after

ovulation, and further increases following implantation of the blastocyst. Among species exhibiting embryonic diapause or delayed implantation, such as Steller sea lions, progesterone is detected after pregnancy but it does little to help researchers determine actual pregnancy rate until gestation is half over (i.e. post diapause). Alternatively, chorionic gonadotropin is a protein hormone produced by the trophoblast of the embryo following fertilization (Sutton-Riley et al. 2006; Cheng et al. 2004; Acevedo 2002). Because chorionic gonadotropin is only present after conception, we propose to develop assay methodologies for the detection of this hormone in the biological fluids (e.g. serum and urine) of Steller sea lions. The ability to measure this hormone has the potential to allow a single sample pregnancy diagnosis during the pre-implantation phase of pregnancy among free-ranging animals, and allow for an accurate estimate of pregnancy rate and reproductive success in this species far in advance of implantation of the embryo.

**Lactation:** Research task 4.1.3 of the Draft Steller sea lion Recovery Plan (NMFS 2006) identified the need to test immune system function, while task 4.2.3 identified the need to examine samples from captive animals to establish baseline endocrine concentration levels. The immune system protects the body from potential infections and is critical for individual health and survival. The mammalian immune system is not fully developed at birth so supplementary antibodies are received via maternal colostrum until their immune system matures. Any delays or suppression of immune system development can lead to long term impaired cell-mediated immune response, which has been associated with lowered T lymphocyte counts and reduced response to mitogens (Chandra 1991, 2002). A compromised cell-mediated immune system would lead to an increase in susceptibility to disease and ultimately mortality.

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 season, 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 (e.g. pregnancy and lactation) and periods of rapid development and growth. For example, a stressed female may produce a pup with low birth weight or could inadequately transfer immune protection to that pup. At this time, it is not clear what normal hormone levels are during gestation, nor is it clear how various hormones affect immunological function during lactation.

**Captives:** Research task 2.5.4 of the Draft Steller sea lion Recovery Plan (NMFS 2006), identifies the need to acquire more knowledge about specific energetic requirements of sea lions during different life stages including periods of rapid growth, pregnancy and lactation. It is imperative that we understand the physiological status in normal, healthy animals before we can identify deviations from the norm and learn their implications for conservation strategies applied to free-ranging Steller sea lions. Although captive animals are not entirely "normal" when compared to wild populations, with the advances of our understanding of nutritional, psychological, and physiological requirements of animals, we can call captive populations in responsible institutions physically and, to some degree,

psycho-sociologically, “normal”. Captive populations, therefore represent an enormous reservoir of information about the basic biology of their free-ranging counterparts.

The 13 year old Steller sea lions held at ASLC have several key advantages over captive animals maintained at other institutions. They have been habituated to daily human physical contact and have been conditioned to voluntarily submit to basic measurements of their health and condition since they were approximately one month old. Researchers have collected physical and hormonal development data on these animals into adulthood (Harmon et al. 1999; Litz et al. 2005). These animals provide a unique study opportunity because they are reproductively intact, sexually mature, and in their prime. Researchers can allow these animals to copulate in order to study the intricacies of “normal” gestation and lactation and then compare it to the long term dataset on these animals as well as their free-ranging counterparts. At the same time, our collection is small for scientific studies. Therefore, we propose to increase our sample size by expanding our collaboration with Mystic Aquarium, Oregon Coast Aquarium, and Vancouver Aquarium.

**Objectives and Justification**

Objectives: We propose to impregnate our captive females, through natural means, in order to study the physical, metabolic, hormonal, and immunological changes expressed during gestation and lactation. We will follow these processes through collection of morphometrics, blood, feces, urine, and milk, and comparison of these data to wild populations. We also wish to retain the authority to re-impregnate our captive females following parturition to evaluate the effects of parturition in consecutive years. Finally, we wish to add all offspring to this research permit so that they may participate in valuable scientific studies.

Hypothesis: Physiological measures of endocrine, immune, and metabolic systems, as well as morphometrics, can be quantified to predict health of an individual.

Justification: ASLC captives are the ideal surrogate for wild animals because they are highly trained for stress-free, voluntary participation in this very type of research. In addition, ASLC researchers are in the unique position of having a large dataset of background information about these animals which covers most of their lives. This information can then be compared to results obtained in this study and later to similar data obtained from wild populations. Finally, any progeny produced by this study will add to the pool of captive animals available for further scientific studies providing valuable life history information.

## C. Methods

**Duration of Project and Location of Taking:** We request this permit be valid for taking of captive Steller sea lions at the Alaska SeaLife Center for 5 years from the final approval date.

Transfer of animals: Up to 1.3 (i.e. 1 male and 3 females) additional adult animals may be transferred from Mystic and/or Oregon Coast Aquarium under current memorandums of

understanding (MOU's), and/or imported from Vancouver Aquarium. The Alaska SeaLife Center and Mystic Aquarium have already established a MOU to promote research and educational ventures, including reproductive studies of Steller sea lions (Appendix B). Similar collaborative efforts aimed at studying Steller sea lion physiology already exist amongst the only North American facilities permanently housing Steller sea lions: the Alaska SeaLife Center, Mystic Aquarium, Oregon Coast Aquarium, and Vancouver Aquarium. In the future, up to 4 animals could be transferred from ASLC to any of these organizations under existing MOU's or new agreements.

There is a well established history of transferring permanently captive Steller sea lions among these organizations. The current ASLC collection was imported from Vancouver Aquarium in 1998 under Permit 881-1443, and Mystic Aquarium has recently loaned 2 neutered males to Oregon Coast Aquarium. Vancouver Aquarium has several females of breeding age that would be appropriate to expand our female sample size, and they may be interested in transferring them to ASLC under a future permit. Following the completion of the ASLC breeding effort, Mystic Aquarium has indicated that they could accept up to 3 same sex juvenile or adult Steller sea lions.

*Breeding:* All Steller sea lions held at ASLC are sexually mature and well within normal age range for breeding, although they have not yet been successfully bred. Females have demonstrated seasonal estrogen and progesterone elevation associated with follicular activity and ovulation, while the male ("Woody") has shown the seasonal testosterone elevation and physical bulk of a territorial bull within the past two years. Male testosterone levels have also been affected by manipulation of female proximity (Beate Litz, unpublished data). We anticipate first attempted breeding to occur during summer 2007 and parturition to occur the following summer. Animals may be allowed to copulate in consecutive years, if one or more were previously unsuccessful, and a total of 4 offspring may be produced. If physical or social factors prevent "Woody" from impregnating the captive females, another male ("Kodiak" NOA0006073) may be transferred from Mystic Aquarium for the following breeding season. All offspring will be permanently captive for research purposes.

*Breeding Location:* All breeding will take place in the ASLC outdoor lab (ODL) or the Steller Sea Lion Habitat (SSLH). The ODL is a roughly 68' x 73' USDA/APHIS inspected and approved, fenced concrete pad comprised of 3 separate enclosures (ODL 6-8). Each enclosure contains a saltwater pool and at least 2 remotely operated exits (Appendix C). When all ODL doors are left open, animals can freely access all 3 enclosures, thereby ensuring that one sea lion cannot trap another simply by blocking the only avenue of escape. The ODL design is flexible enough that it can house a large group of breeding animals, and could even accommodate 2 sexually mature males simultaneously. The SSLH includes the habitat ASLC guests see, as well as a holding pool and vestibule they cannot (Appendix C). The SSLH is designed to limit the access of the male sea lion to the main exhibit pool while allowing the females access to all pools and holding areas. Every gate between each enclosure and holding area is remotely operated and the overall SSLH design allows for animals to be moved amongst all areas without having contact with each other.

Current Alaska SeaLife Center captives have been sufficiently desensitized to one another through a lifetime of proximity and contact that they routinely occupy the same or adjacent enclosures. In anticipation of safely breeding these animals, they are being taught to respond to a recall signal. Recall signals are routinely trained in public display animals at interactive facilities to provide a safe environment for animals and guests alike. When the recall signal is given, the animals stop what they are doing and return to a pre-assigned station (i.e. separate enclosures). ASLC captives are highly trained, eager participants, and we are confident that they will respond to a recall signal regardless of circumstances.

The captives will be kept within the same enclosures (i.e. outdoor lab or Steller sea lion habitat) throughout the breeding season to maintain their familiarity and social dynamics, and increase the likelihood of successful copulation. All doors will be opened in the presence of Husbandry staff, and animals will be allowed to occupy the area of their choosing. Animal behavior will be closely monitored to ensure that no aberrant interactions occur. Security cameras may be used to record behaviors for later review. Animals will be separated when Husbandry staff members are no longer present, unless their behavior has been consistently ideal such that Husbandry staff, P.I., and Attending Veterinarian are comfortable leaving them together.

*Emergency Separation:* ASLC Husbandry staff is accustomed to "normal" and "abnormal" behaviors, and will respond appropriately to any animal exhibiting a pronounced deviation from a normal healthy state. If an undesired behavior (e.g. sustained unwelcome advances, repeated biting producing puncture wounds, etc) is observed, the recall signal will be given and animals will be separated. If one or more animals delay in returning to their station, or continue with the undesired behavior, the recall signal will be given a second time. If the second recall signal fails to separate them, various forms of sensory distraction will be used until they have been separated. Once animals are separated, Husbandry staff will summon the Attending Veterinarian and P.I. and the health of the animals will be examined. Animals will be allowed to associate with conspecifics again at the discretion of the Attending Veterinarian and the Mammal Curator.

*Copulation:* Free-ranging female Steller sea lions typically copulate only once during the 2-5 days that they are receptive (John Maniscalco, personal communication). Free ranging males typically test for receptiveness by smelling/licking female genitalia, and females often indicate receptiveness by biting the male's neck while presenting her posterior. Following an observed copulation, the recall signal will be given, animals will be separated, and voluntary vaginal swabs will be taken and assessed for presence of motile sperm. Since all sexually mature wild females are typically impregnated in any given year (Pitcher and Calkins 1981), we presume that the presence of motile sperm will be a good indicator of impregnation. However, because a small percentage of females copulate more than once, we will allow our animals to remain together following copulation, dependant upon good behavior. If aggressive behavior is observed at this time, animals will be separated. Male and female sea lions will be housed separately following the breeding season.

*Confirmation and Monitoring of Pregnancy:* Since Steller sea lions have an embryonic diapause, tests examining fecal concentrations of progesterone are unable to confirm pregnancy until at least December. Alternatively, among other species, chorionic gonadotropin is indicative of pregnancy within weeks of fertilization. Researchers at ASLC will develop assays to test for concentrations of chorionic gonadotropin in bodily fluids (e.g. blood and urine) routinely collected during other research activities, which in turn will confirm pregnancy months earlier than previous methods.

Veterinary and Research staff will monitor follicular and fetal development utilizing ultrasonography. Ultrasonography is an imaging technique in which deep structures of the body are visualized by recording the reflections of ultrasonic waves. It is an effective diagnostic tool for the examination of a variety of organs and the identification of size and structural changes. Ultrasound is particularly useful in assessing reproductive status and diagnosing pregnancy or reproductive disorders (Adams et al. *in press*.).

Portable ultrasound technology has advanced significantly over the past decade to the extent that current production of instruments (e.g. Sonosite 180Vet model- Sonosite, Inc) are being utilized in field situations (Adams et al. *in press*.). Light pressure required for transducer to skin interface produces little or no reaction from alert, targeting, long-term captive animals at the ASLC (Pam Tuomi, personal communication). In addition, multiple readings can be obtained within a minute and stored images of these readings can be analyzed at any time with basic imaging software (e.g. Photoshop).

Full and detailed visualization of structures deep in the abdominopelvic cavity may require the use of rectal or vaginal transducers. During transrectal or transvaginal ultrasonography, a well lubricated transducer probe is inserted in the appropriate orifice to the minimum depth required to fully visualize the structures being observed. Level of restraint or sedation necessary during transrectal or transvaginal ultrasonography is at the discretion of the Attending Veterinarian.

*Parturition:* Parturition among free-ranging Steller sea lions within the Gulf of Alaska typically occurs in late May through June. As pupping season nears, captive pregnant females will be kept within the Steller sea lion habitat (SSLH). All gates within the SSLH are remotely operated and can be used in such a way that animals can be shifted to different areas without having contact with each other. At night, females will be separated into individual holding areas that have been designed to allow them access to water while ensuring any pups would stay within a dry holding area. Veterinary staff members are always on call 24hours should any complications arise during birth or any other time.

During the first week, postpartum mother pup pairs will be closely monitored to ensure that pups are nursing. If nursing is not observed within 24-48 hours, the pup will be removed so that ASLC staff can begin feeding it according to protocols developed by the veterinary and rehabilitation staff. After the pup is stabilized, an attempt will be made to reintroduce it to the mother to establish a normal mother-pup bond. Should reintroduction attempts fail, ASLC staff will rear the pup by hand until such time that it can be introduced to the SSLH.

Pups will be allowed supervised access to a small wading pool at one week of age. At approximately two weeks of age, pups will have supervised access to deeper water until they demonstrate the ability to enter and exit the pool unassisted. After several weeks of incident free deep water access, they may be granted access to the entire Steller sea lion habitat.

*Consecutive breeding:* The ASLC captives are naïve animals (i.e. have not previously been bred) and we anticipate a higher likelihood of breeding failure in the first year. Since the proposed study cannot be performed on non-pregnant animals, we intend to continue breeding in consecutive years until successful. Since Mystic Aquarium has expressed interest in acquiring any combination of up to 3 females or neutered males from ASLC, we propose to continue breeding captives until a maximum of 4 offspring are produced.

*Anesthesia during gestation and lactation:* Isoflurane is a halogenated volatile anesthetic that has been safely used for decades on a variety of animals, including those that are pregnant. Likewise, ASLC Veterinary staff regularly uses isoflurane anesthesia during the handling of ASLC Steller sea lions for research and health assessment procedures. Because of its low solubility in tissues and bodily fluids, isoflurane is quickly eliminated from the body via the lungs when administration is stopped, and is not thought to be significantly excreted through lactation (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 these mother-pup pairs at Lowrie Island in Southeast Alaska. Therefore, we feel that continued use of isoflurane is justified, regardless of gestation or lactation status.

*Research during gestation and lactation:* Steller sea lions are maintained at the Alaska SeaLife Center for highly important research purposes. Impregnation does not preclude them from this research; rather it makes the work all the more vital. In fact, we believe that up to 9 recovery tasks in the 2006 Steller Sea Lion Draft Revised Recovery Plan will be addressed by this work. Therefore, captive females will continue to participate in permitted research projects deemed harmless to mother, fetus, and pup by the Attending Veterinarian, the Husbandry Director, and the Principle Investigator. However, upon successful impregnation, we expect to discontinue the following research on pregnant animals: ACTH and TSH challenges and fasting studies. Moreover, fasting studies will not be performed on lactating animals.

*Milk collection:* Husbandry staff will train animals to allow collection of milk samples under behavioral control. Samples may also be collected under mild restraint or general anesthesia during other procedures. The skin and hair around the nipple will be cleaned with a dilute disinfectant prior to collection. Small amounts of milk (i.e. several ml) can be expressed into a clean vial through gentle manipulation of the nipple(s), but larger volumes will likely require the use of a suction device (e.g. plastic syringe or human breast pump). Oxytocin (IM, IV 20 – 40 USP Posterior Pituitary Units or as a nasal spray 5-10 minutes before the procedure) may stimulate milk letdown and assist in collection of larger volumes of milk. Volume of milk collected is not to exceed 100ml per day. This maximum volume may be the result of several smaller samples collected, under behavioral control,

throughout the day. Frequent collection of smaller samples will allow for more finite monitoring of changes in milk composition.

*Feces and Urine:* Additional fecal and urine samples are needed throughout the year to accurately monitor hormonal cycles. Currently, we are permitted to collect fecal or urine samples opportunistically (e.g. after natural deposition) which does not meet current research and health monitoring needs. Therefore, Husbandry staff is training these animals to voluntarily accept the insertion of a well lubricated fecal loop into the rectum and withdrawal of a small fecal sample. We request permission to also utilize this husbandry technique to obtain samples for current research objectives. If we are unable to obtain voluntary samples, animals will be held in separate enclosures for short periods until a sample is deposited naturally. In instances when separation is not feasible (e.g. breeding season) we propose to include biomarkers such as kernels of canned corn within their food to individually identify fecal samples deposited naturally.

*Pups:* Any pups produced during this study will play an important and evolving role in fulfilling the ASLC research mission. As such, we request that they be added to this permit. ASLC Husbandry staff will begin training pups, at their discretion, to voluntarily present various body parts for several noninvasive procedures. Much like their mothers, they will be trained to calmly sit on a platform scale while body mass is measured, allow for measuring equipment to be placed around them for morphometric measurements (e.g. standard length, curvilinear length, and several girths), and remain still while Veterinary or Research staff use a portable ultrasound machine to measure blubber thickness. In addition, pups will be photographed by staff using several types of cameras (e.g. digital, video, thermal, spectrophotometric, etc), and vocalizations will be recorded utilizing basic audio recording equipment placed in the vicinity. After Husbandry and Research staff, and the P.I., are comfortable that a stable mother-pup bond has been established, routine health and condition assessments will begin. Feces and urine may be collected opportunistically or under mild restraint or general anesthesia during other procedures. Blood will be drawn as per ASLC protocols submitted with previous permit applications.

*Expected Results:* The requested study will produce valuable baseline data, in the form of a known standard, which can be integrated into population models developed by wildlife managers to better understand the depleted Steller sea lion populations. Any information that can assist manager's assessment of free ranging Steller sea lion pregnancy and parturition rates will improve predictions of future population dynamics while addressing high priority recovery tasks (e.g. Draft Steller sea lion Recovery Plan, NMFS 2006). Research on pups will supply crucial long term data about a segment of the population once thought to be disproportionately represented in the overall decline of western stock Steller sea lions. These studies may ultimately be used in modeling efforts on the growth and development of young Steller sea lions.

**Types of Activities, Methods, and Numbers of Animals or Specimens to be Taken:**

**Table 1.** Annual take table for all research on Steller sea lions held at Alaska SeaLife Center. For the purpose of clarity, takes are listed in two main categories: those involving adults/juveniles, and those that involve pups. When pups reach 12 months of age, research will be performed under the Adult and Juvenile takes. All takes currently permitted for ASLC captives are listed. Unless otherwise noted, all research listed in the official take table will be performed upon all adults, including those transferred from cooperating institutions. Where reference is made to Projects I-V, please refer to the official take table issued with Permit 881-1745.

| **Table 1. Annual takes for captive Steller sea lions identified in this amendment request. Takes are described for each project, with some take activities overlapping among existing projects. Takes described for multiple projects are "piggy-backed" to the maximum extent practicable.** | | |
|---|---|---|
| **Project and Activity/Take** | **# Takes/ Animal/ Year** | **Notes** |
| **ADULTS & JUVENILES AGED >12mo.** | | |
| Project I: Condition Assessment of juveniles and adults >12months old | | |
| Project II: Endocrinology and Immunology Study | | |
| Project III: Assessing Metabolism in Steller sea lions: Implications for Energetic and Digestive Costs at Sea | | |
| Project IV: Metabolic Demands of Steller sea lion Survival | | |
| Project V: Biotelemetric Monitoring of Foraging Behavior | | |
| ***Project VI: Physiology of Gestation and Lactation*** | | |
| 1. Measure body mass and morphometrics | Up to daily under behavioral control | All Projects |
| 2. Blubber depth measurements using ultrasound | Up to daily under behavioral control | Also used in Projects I, ***II*** & III |
| 3.a. Routine Blood Samples: total volume per month (including other studies requiring blood samples) not to exceed 5% calculated blood volume based upon animal body mass at time of sample | Once every ***calendar*** month, up to 12x/year[1] **Procedures are separated by a minimum interval of 21 days.** | Also used in Projects I, II, & IV |
| 3.b. Additional Blood Samples: total volume per month authorized in 3.a | Once a week for up to 4 consecutive weeks, no more than 4x/year; of the 4 consecutive weekly samples, one sample must include a monthly sample from 3.a[1] | Also used in Projects I, II, & IV |
| *4. Urine, feces ,whiskers,* **and milk.** | *Urine, feces,* ***and milk****: up to daily if collected opportunistically after natural excretion,* ***under behavioral control, or when under restraint or anesthesia for other procedures.*** *Whiskers:* | Also used in Projects I, II, & III |

| | | |
|---|---|---|
| | *two vibrissae pulled 4x/year*[2] | |
| 5. Bio-electrical Impedance Analysis (BIA) | Once every month, up to 12x/year while under anesthesia | Also used in Projects I, ***II***, & IV |
| 6. Total Blood Volume (TBV): initial ~~3 ml~~ blood draw followed by up to 0.5 mg/kg injection of Evan's Blue Dye; ~~3 ml~~ blood samples drawn ~~10 and 20 minutes~~ ***twice*** after injection | Once every 4 months, up to 3x/year | Also used in Project I |
| 7. $D_2O$ Administration: ~~2 ml~~ pre-administration blood sample followed by $D_2O$ administration (dosage up to 0.7g/kg + 10% IM); ~~2ml~~ blood sample at 2 hours post $D_2O$ injection. Once yearly for validation purposes, additional blood sample at 1 hour post $D_2O$ injection is collected | Once every month, up to 12x/year | Also used in Projects ***II***, III, & IV |
| 8. Nutritional Physiology: Food trials, dietary manipulation (includes live fish ***and dietary markers***) | Diet change to be determined; fasting or caloric restriction not to exceed 14 days 4x/year[3] | Also used in Projects I, IV, & V |
| 9. Epidermal and mucosal swabs and collections of saliva and other secretions; examine and measure external genitalia | Up to ~~3x/ week~~ ***daily*** under behavioral control or when under restraint or anesthesia for other procedures | Also used in Projects I ***& II*** |
| 10. Blubber biopsies (up to 2g/sample) | Up to 6 x/year while under anesthesia[4] | Also used in Projects I ***& II*** |
| 11. Imaging: Video, photographic, radiographic, spectrophotometric, digital, and thermal imaging of animals | Up to daily under behavioral control, or when under anesthesia for other procedures | All Projects |
| 12. Radiographic examination | Up to daily when conducted under behavioral control or when under anesthesia for other procedures.<br><br>Total annual exposure not to exceed the safe limits for human radiation workers for total effective dose equivalent to the whole body, which is 5,000 mrem (50 mSv) per year. | Also used in Projects I & II |
| 13. Hormone Stimulation: ACTH (2 IU/kg) or TSH (0.1 IU/kg) administration + post dosage samples; potential dry holding for up to 4 days for post dosage fecal ***and blood*** sample collection | 3x/year[5] | Also used in Project II |
| 14. Attachment and removal of instrumentation (recorder, sensors, monitors, etc.) using various attachment methods (epoxy, harness, etc.) | Up to daily under behavioral control; 2x/month if restrained/anesthetized | Also used in Projects III, IV, & V |

| | | |
|---|---|---|
| 15. Underwater foraging and drag trials | Up to daily under behavioral control | Also used in Projects III, IV, & V |
| 16. Bioenergetics: determine resting and active metabolic rate, heart and breathing rate, flipper stroke frequency, body temperature and heat flux using chambers, cages, pools, etc. | Up to daily under behavioral control[6] | Also used in Projects ***II*** & III |
| 17. DLW Validation: initial 10ml blood sample followed by injection of DLW ($D_2O$ and Oxygen-18; 1ml/kg body weight); post-injection blood samples at 1, 2, 4, and 6 hours as described in application: followed by dry-holding up to 96 hours for simultaneous respirometry | ~~2~~ ***4***x/year[7] | Also used in Projects ***II*** & III |
| 18. Bioenergetics and Metabolic Development: Dietary marker administration + dry holding for up to 72 hours for post dosage fecal and urine sample collection | 4x/year[8] | Also used in Project III |
| 19. Protein Turnover: Stable isotope and tissue metabolism: ingestion or IV administration of stable isotope $^{13}C$ and $^{14}N$ and post dosage blood sampling (serum samples collected approximately at 3 hours, 1 day, 2 days, 5 days, 10 days, 20 days: and then monthly blood samples taken concurrent with "Condition Assessment" project; sampling times/days may vary slightly to allow for piggy backing with other projects to minimize handling/sampling frequency, but will not exceed the number of samples listed above) | Once every 4 months | Also used in Project III |
| 20. Stomach temperature telemetry | Up to daily under behavioral control, or when under anesthesia for other procedures | Also used in Project V |
| 21. Gas anesthesia | As deemed necessary by Attending Veterinarian and in coordination with other projects | All projects |
| *22. Transrectal ultrasonography* | *Up to daily under behavioral control, or when under restraint or anesthesia for other proceedures*[9] | |
| *23. Transfer of animals currently housed within Aquariums in the North America* | *Up to 1.3 animals*[10] | |
| *24. Copulation & Parturition* | *Via natural means*[11] | |
| ***PUPS*** | | |
| ***Project VII: Condition assessment of pups aged 0-12 months*** | | |
| *1. Measure body mass and morphometrics* | *Up to daily under behavioral control* | *All projects* |

| | | |
|---|---|---|
| *2. Blubber depth measurements using ultrasound* | *Up to daily under behavioral control* | *All projects* |
| *3. Routine Blood Samples: total volume per month (including other studies requiring blood samples) not to exceed 5% calculated blood volume based upon animal body mass at time of sample* | *Once every month, up to 12x/year*[12] | |
| *4. Urine and feces* | *Up to daily if collected opportunistically or under behavioral control or when under restraint for other procedures.* | |
| *5. Visual and audio recordings: Video, photographic, radiographic, spectrophotometric, digital, and thermal imaging, as well as audio recordings of pups* | *Up to daily under behavioral control or when under restraint for other procedures.* | |
| *6. Radiographic examination* | *Up to daily when conducted under behavioral control or when under anesthesia for other procedures.*<br><br>*Total annual exposure not to exceed the safe limits for human radiation workers for total effective dose equivalent to the whole body, which is 5,000 mrem (50 mSv) per year.* | |
| *7. Bioenergetics: determine resting and active metabolic rate, heart and breathing rate, body temperature and heat flux using chambers, cages, pools, etc.* | Up to daily under behavioral control[13] | |
| *8. Gas anesthesia* | *As deemed necessary by Attending Veterinarian and in coordination with other projects* | |

[1] Routine blood sampling may occur once every month ***(i.e., once every calendar month. Consecutive routine blood sampling will be separated by a minimum interval of 21 days)***; weekly blood sampling (i.e., once every 7 days), which is not routine, may occur around dietary changes ***or significant reproductive events (e.g. estrus, end of embryonic diapause, etc)***, no more that 4 times per year for up to 4 consecutive weeks, followed by at least 30 days before another round of weekly sampling occurs. Where weekly sampling occurs, one monthly sample must be used for one of the consecutive weeks (i.e., the monthly take will count as one of the weekly takes, for no more than 4 samples taken in a month, and the total number of samples may not exceed 24 per year).

[2] ***Urine, feces, and milk to be obtained daily if collected opportunistically or under behavioral control. Feces and urine may also be collected through short term separation of animals until feces are deposited naturally. Feces can also be collected via fecal loop under behavioral control. Volume of milk collected is not to exceed 100ml***

***per day.*** Consecutive whisker removal procedures (2 whiskers each procedure) will be separated by a minimum interval of 14 days.

[3] Consecutive fasting or caloric restriction trials will be separated by a minimum interval of 60 days. ***Fasting studies will not be conducted on gestating or lactating animals.***

[4] Consecutive blubber biopsies will be separated by a minimum interval of 7 days.

[5] Only a total of 3 trials of either ACTH or TSH are authorized per year (i.e., not 3 trials each); consecutive ACTH or TSH trials will be separated by a minimum interval of 30 days. ***ACTH and TSH trials will not be performed on females during gestation.***

[6] Metabolic Measurements
Resting in air up to 2 hours - up to daily ***under behavioral control, or*** 50 times per year;
Diet metabolic variation - up to 72 hours - up to 6x per year;
Heat increment of feeding - up to 12 hours - up to 1x per week not to exceed 24 trials per year;
Resting in water up to 15 minutes - up to daily ***under behavioral control, or*** 50 times per year;
Swimming - up to 2 hours - up to daily ***under behavioral control,*** not to exceed 30 complete trials;
Fasting or caloric restriction - can last up to 14 days - metabolic measurements of the various types listed above would occur throughout the experiment (not exceeding the frequency noted per activity) - 4x per year.

[7] Consecutive DLW validation trials will be separated by a minimum interval of 90 days.

[8] Consecutive dietary marker and associated dry holding trials will be separated by a minimum interval of 14 days.

*[9] Transrectal ultrasonography may occur up to daily if under behavioral control or restraint, but under general anesthesia during other procedures only.*

*[10] Up to 1.3 (i.e. 1male, specifically "Kodiak" NOA0006073, and 3 females) Steller sea lions to be transferred from Mystic and/or Oregon Coast Aquarium, and/or imported from Vancouver Aquarium. Acquired animals will be trained and sampled as captives currently are.*

*[11] Copulation will be through natural coupling of captive animals maintained at ASLC. Behaviors will be monitored to ensure safety of animals involved. Copulation may occur in during consecutive years and may result in multiple offspring from each female. Maximum number of pups produced not to exceed 4. Final disposition of offspring is the Alaska SeaLife Center.*

*[12] Routine blood sampling may occur once every month (i.e., once every calendar month. Consecutive routine blood sampling will be separated by a minimum interval of 21 days)*

*[13] Metabolic Measurements*
*Resting in air up to 2 hours - up to daily **under behavioral control, or** 50 times per year;*
*Resting in water up to 15 minutes - up to daily **under behavioral control, or** 50 times per year;*
*Swimming - up to 2 hours - up to daily **under behavioral control,** not to exceed 30 complete trials.*

**Takes and Mitigation Measures for Steller Sea Lion ADULTS and JUVENILES:**

New or amended takes on 3 adult Steller sea lions (currently held at ASLC) are as follows: blood, urine, feces, milk, and transrectal ultrasound. The takes being requested (e.g. feces, urine, milk, blood) are harmless and largely noninvasive and follow well established protocols. ASLC staff takes great pride in the fact that captives are trained to voluntarily participate in the majority of procedures which greatly reduces stress and discomfort. Whenever possible, research takes are coordinated with husbandry activities in order to minimize handling.

***Breeding***– has been described in detail in the methods section of this permit application.

***Breeding Mitigation:*** Copulation among pinnipeds may look uncomfortable to humans, but it is a natural process that shouldn't cause any ill effects. However, Steller sea lions can and do get injured during breeding activities, in both natural and artificial settings, though fatalities are rare. Both the Research and Husbandry staff have intrinsic knowledge of breeding behaviors, and will be closely monitoring captive interactions. All doors in the ODL will be open so that all animals can intermingle thereby reducing the chance of a single animal being cornered. Further, tires and other structures will be placed within the enclosures to create visual barriers and areas of refuge for an animal attempting to escape. In addition, animals are being trained to respond to a recall signal which requires them to stop what they are doing and go to an individual station. Should an animal become overly agitated or aggressive during the course of breeding, the recall signal will be given and they will be separated. Various forms of sensory distraction may be used to facilitate separation of animals.

***Gestation Mitigation:*** The unborn fetus could potentially be harmed through direct physical trauma to the mother during gestation. Therefore, male contact with females will be kept to a minimum during non breeding times. Increased stress to the mother could also harm the fetus, so stress mimicking research (i.e. TSH and ACTH challenges, fasting studies) will not be performed during gestation.

***Parturition Mitigation:*** ASLC Veterinary staff will be available 24 hours should complications arise at any time, but especially during birth. In the event of a spontaneous abortion or stillborn pup, Veterinary staff will examine it and attempt to determine cause of death. If a pup were rejected, ASLC staff is prepared to hand rear it. Postpartum Steller sea lions are protective of their offspring and can be aggressive toward other animals. For the first week, mothers will only have access to their own pup, and will gradually be introduced to any other mother/pup pairs only when the Attending Veterinarian and Husbandry Director deem it appropriate. These interactions will be closely monitored and animals will be separated if deleterious behavior, such as biting and tossing another's pup, is observed.

***Blood***– collected as per previous ASLC research applications submitted to Office of Protected Resources. Samples will not exceed 5% calculated blood volume based upon animal body mass at time of sample.

Routine blood sample volumes can vary, but this is the typical requirement:

10ml blood- Veterinary diagnostics: hematology and blood chemistry
5ml blood- Serology: bacteriology and virology
40ml blood- Endocrinology: includes assays of free and bound triiodothyronine (T3) and thyroxine (T4), cortisol, corticosterone, testosterone, progesterone, estrogen, leptin and ghrelin, melatonin, serotonin, aldosterone, and the development of a chorionic gonadotropin assay
40ml blood- Immunology: immunoglobulin assays, innate and acquired immune assays (e.g. lymphocyte proliferation, phagocytosis, respiratory burst, etc), cytokines and normal hematology including differentials
20ml blood- Contaminants: organochlorines, PCBs, PDBEs, DDTs, PCNs, mercury, pesticides
20ml blood- Archive: for future, retrospective studies

***Mitigation:*** Other non-routine blood sample requirements are dependent upon the experimental protocol, but whenever possible, these blood draws are scheduled to coincide with routine sampling in order to minimize the total amount of sea lion handling and the number of needle insertions required. The most common site for blood collection in sea lions is the caudal gluteal vein, which is near the animal's tail, just to the side of the spine. Flipper veins are frequently used too. For procedures that require multiple blood draws over a short period, we will catheterize a flipper vein or the caudal gluteal vein to eliminate multiple venipunctures.

***Urine and Feces***– Whenever possible, feces and urine are collected opportunistically after natural excretion or under general anesthesia during other procedures. However, various veterinary health assessments and research objectives require more consistent and frequent fecal samples than we are currently obtaining. Therefore, husbandry staff is training animals to accept the insertion of a well lubricated fecal loop into the rectum for the voluntary collection of fecal samples. Further, animals may be held separately for short periods of time until a fecal sample is deposited naturally. During periods when separation is not possible (e.g. breeding season), dietary markers, such as kernels of corn, may be added to their food supply thereby making fecal samples individually identifiable.

***Mitigation:*** Collection of feces through voluntary fecal loop has been used in other species of pinnipeds and cetaceans with no ill effects. ASLC staff will observe standard fecal collection protocols and will also ensure that fecal loops will be thoroughly cleansed and disinfected between individuals to avoid transmission of zoonotic agents. No ill effects are expected from short periods of separation for the purpose of collecting fecal samples deposited naturally. Canned corn utilized as a dietary marker to individually identify feces, will be fit for human consumption and are expected to pass undigested through the gastrointestinal tract.

***Milk***– Animals may be trained to allow collection of milk samples under behavioral control, or under general anesthesia during other procedures. The skin and hair around the nipple will be cleaned with a dilute disinfectant. Small amounts of milk can be expressed into a clean vial through gentle manipulation of the nipple, but larger volumes will likely require the use

of a suction device (e.g. plastic syringe or human breast pump). Routine milk collection volumes may vary but will not exceed 100ml per day.

***Mitigation:*** Milk samples will be obtained under behavioral control whenever possible or in conjunction with other research being performed. Oxytocin, a naturally occurring hormone that stimulates milk letdown, (IM, IV 20 – 40 USP Posterior Pituitary Units or as a nasal spray 5-10 minutes before the procedure) may assist in collection of larger volumes of milk. The Attending Veterinarian and Mammal Curator will ensure that research needs (up to 100ml milk/day) do not usurp pup dietary needs. If they determine that pups are not receiving adequate volumes of milk, it may be necessary to temporarily reduce the volume of milk available for research.

***Transrectal Ultrasound***– Ultrasonography is already being used on these animals to visualize deep structures of the body through reflections of ultrasonic waves. However, assessing reproductive status and diagnosing pregnancy or reproductive disorders may require the use of a transrectal approach. This procedure may be performed under behavioral control or restraint, or sedation may be necessary for the comfort of the animal. Species and individual anatomy will be considered when selecting a probe of appropriate length and diameter. The probe and associated extensions will be well lubricated prior to insertion. The probe will be advanced to the minimum depth necessary for structural visualization and this depth will be recorded.

***Mitigation:*** The transrectal ultrasound will only be used by, or under direct supervision of, experienced personnel or Veterinary staff. Animals will only be restrained to the level which is deemed necessary by the mammal curator and attending Veterinary staff. The probe will be well lubricated, and care will be taken to avoid introducing foreign matter into the vaginal canal.

**Takes and Mitigation Measures for Steller Sea Lion PUPS Aged 0-12 Months:**

New takes on any Steller sea lion pups produced during research are as follows: morphometric measurements, metabolic measurements, ultrasound, visual and audio recordings, blood, urine, and feces. All research procedures will be gradually phased in to ensure that there is no disruption to the mother-pup bond.

Wild pups frequently experience extended separation and fasts while their mother is foraging. ASLC pups however, will have daily access to their mother unless a particular short term research objective dictates otherwise. Also, a short fast may be necessary at 9-12 months of age when we are weaning them prior to the next pup being born.

No ill effects are anticipated from the collection of feces, urine, morphometric or metabolic measurements, ultrasounds, or from visual or audio recordings of pups due to the noninvasive, voluntary nature of the work. Both blood samples and metabolic measurements will be collected utilizing the same protocols used for years on our captive adults.

***Morphometric Measurements***– Pups will be trained to calmly sit on a platform scale while body mass is measured. They will also be trained to allow various measuring equipment to

be placed around them so that we may obtain standard length, curvilinear length, and several girths. The voluntary nature of the procedure ensures stress will be kept to a minimum.

***Metabolic Measurements***– Pups will be trained to voluntarily enter a metabolic chamber and remain still while metabolic measurements are obtained, as per protocols submitted with previous ASLC applications. The voluntary nature of the procedure ensures stress will be kept to a minimum.

***Ultrasound***– Pups will be trained to remain still while Veterinary or Research staff uses a portable ultrasound machine to measure blubber thickness. Blubber will be measured from multiple sites, including the xiphosternal, neck, shoulder region, and hind quarters following ASLC IACUC approved protocols. Ultrasound of the abdomen, chest, and extremities will be used to assess organ size and growth, and cardiac function. The voluntary nature of the procedure ensures stress will be kept to a minimum.

***Blood***– Blood will be collected as per ASLC collection protocols utilized on the adult captives as well as in the field. Samples will not exceed 5% calculated blood volume based upon animal body mass at time of sample. Isoflurane gas may be used at the discretion of the Attending Veterinarian to minimize any distress. Whenever possible, blood draws are scheduled to coincide with routine sampling in order to minimize handling.

***Visual and Audio Recordings***– Video, photographic, radiographic, spetrophotometric, digital, and thermal images of pups, as well as audio recordings, will be taken under behavioral control. The voluntary nature of the procedure ensures that stress will be kept to a minimum.

***Urine and Feces***– Feces and urine will be collected opportunistically after natural excretion. The voluntary nature of the procedure ensures that stress will be kept to a minimum.

**Additional Information for Research or Enhancement on Captive Animals**

Three Steller sea lions taken under this permit are permanent captives maintained by the Alaska SeaLife Center. This includes “Woody” (NOA0005799), “Kiska” (NOA0005800), and “Sugar” (NOA0005801). This permit request also includes the transfer of a permanent captive (“Kodiak” NOA0006073) from Mystic Aquarium to ASLC. Additional permanent captive Steller sea lions taken under this permit include up to 4 pups produced during the proposed study, as well as up to 3 adult females transferred from Mystic and/or Oregon Coast Aquarium, or imported from Vancouver Aquarium.

All animals participating in or resulting from this study will be kept at ASLC in perpetuity, or may be transferred to Mystic and/or Oregon Coast Aquarium under existing agreements, and/or imported by Vancouver Aquarium. We would like to reiterate that ASLC and Mystic Aquarium already have a MOU, that Mystic Aquarium has already verbally committed to accepting up to 3 females or neutered males, and that other Cooperating Institutions have also expressed interest in accepting animals.

The Alaska SeaLife Center is a USDA-APHIS licensed and inspected facility (Appendix D) with documents detailing physical descriptions of facilities and techniques on file with

Office of Protected Resources or provided within this application. Our facilities and protocols are appropriate for the study requested, and our staff members are well qualified to ensure the highest quality animal care available (Appendix E).

**Lethal Take**

Our sea lions are important ambassadors for the Alaska SeaLife Center and the community of Seward alike, and as such, great care is always taken to ensure that any potential for injury has been mitigated. The proposed study does not require sacrificing animals to accomplish any research objectives. However, we recognize that there are risks associated with breeding and rearing captive animals, and therefore request one (1) lethal take of a live-born Steller sea lion during the proposed study. Stillborn or spontaneously aborted pups are natural occurrences among free-ranging Steller sea lions that occur for unknown reasons. Therefore, if a stillborn or spontaneously aborted pup occurs during this study, it will not be counted as a lethal take, though every effort will be made to determine what caused the condition.

In the event of a serious injury or lethal take, research activities will halt until qualified personnel can review the incident. In that event, all procedures will be evaluated and Veterinary staff will attempt to identify the cause of mortality. This will be a thorough review and will take as much time as required. 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). Procedures will be adjusted as necessary to prevent a reoccurrence.

**Measures to minimize effects**

The captive animals that are studied under this permit receive excellent husbandry and veterinary care, and are maintained at standards above USDA AWA regulations. Cumulative effects are monitored and reviewed by a trained staff of marine mammal husbandry specialists, researchers, and veterinarians. The research program and the veterinary care programs are overseen by an Institutional Animal Care and Use Committee, as well as a Scientific Advisory Committee. Research studies are “piggy-backed” whenever possible to minimize handling and stress.

**Monitoring effects of activities**

The ASLC Husbandry staff monitors animals daily, throughout working hours (i.e. 0800 to 1700), 7 days per week, and Veterinary staff perform frequent health assessments as part of their regular care for the animals. Round the clock monitoring is available at any time, and may be utilized during breeding and pupping seasons.

**Alternatives**

It would be impossible to accurately describe the physiology of gestation and lactation in adult female Steller sea lions through any methods that did not involve impregnation. Natural breeding has a high success rate in many species of captive pinnipeds, and does not require much additional handling on our part. Considerable thought has been given to artificial insemination (AI) as a way to eliminate some of the risks associated with potential male aggression during natural breeding, but we believe that AI techniques (e.g. electro-

ejaculation, insemination, etc) should be reserved, lest natural impregnation is unsuccessful.

**IACUC**

As per ASLC policy, protocols for intrusive animal research must be reviewed and approved by the ASLC IACUC committee, and a copy of the approval letter must be on file at the Permits, Conservation and Education Division. The current IACUC Approval Letter is on file with OPR.

## E. Resources Needed to Accomplish Objectives

Not applicable

## F. Publication of Results

The Alaska SeaLife Center is developing an impressive record of peer-reviewed publications in international journals. In addition, new and innovative research findings are presented at numerous annual conferences, workshops, and seminars. Moreover, research findings and discoveries are distributed to ASLC staff and the general public through our research education program, as they become available.

## *V. National Environmental Policy Act Considerations*

**1.** Will your research or enhancement activity involve equipment (*e.g.*, scientific instruments) or techniques that are new, or may be considered innovative or experimental? If yes, are they likely to be adopted by other researchers in the future?

**The proposed research is a suite of behaviors and tasks that are routinely performed on captive animals in zoos and aquariums around the world. While the intent might be different, captive management vs. research data production, the actual procedures are the same or similar. Because the Alaska SeaLife Center is becoming known as a leader in captive animal research and management, any new techniques developed during this study are likely to be adopted by the broader scientific community.**

**2.** Does your activity involve the collection, handling, or transport of potentially infectious agents or pathogens (*e.g.*, biological specimens such as blood), and/or does your activity involve the use or transport of hazardous substances (*e.g.*, toxic chemicals)? If so, provide a description of protocols to be used to ensure human safety from injury or zoonotic disease transmission.

**ASLC protocols for collecting and handling biohazard materials are on file with OPR.**

**3.** If any of your activities occur in or near unique geographic areas (such as National Marine Sanctuaries, Marine Protected Areas, State National Parks or Wilderness Areas, Wildlife Refuges, Wild and Scenic Rivers, designated Critical Habitat for endangered species, Essential Fish Habitat, etc.), would any aspect of your activities impact the physical environment, such as by direct alteration of substrate (*e.g.*, bottom trawling, net setting, anchoring vessels or buoys, erecting blinds or other structures, disrupting nesting bird habitat, etc.)?

**Not applicable because research is conducted only on captive animals at ASLC.**

**4.** Do you know if your work could affect entities listed in or eligible for listing in the National Register of Historic Places, or cause loss or destruction of scientific, cultural, or historic resources (*e.g.*, archeological resources, species used for subsistence purposes, etc.)? If so, list the sites and explain how they might be affected or why they would not be affected.

**Not applicable because research is conducted only on captive animals at ASLC.**

**5.** Would any of your proposed activities include actions that might involve the transportation of any material, biological or otherwise, from one area to another (*e.g.*, transport of animals or tissues, ballast water discharge, working in sensitive remote areas, etc.). If so, please explain the types of activities and indicate any measure you would take to prevent the possible introduction or spread of non-indigenous or invasive species (including plants, animals, microbes, or other biological agents).

**Not applicable because research is conducted only on captive animals at ASLC.**

## *VI. Previous and Other Permits*

**Previous Permits**

NMFS Permits 881-1443, 881-1668, 881-1710, 881-1724. All reports have been submitted.

General Authorization for Scientific Research for Level B harassment. Letter of Confirmation No. 881-1673. Expiration July 31, 2007. Annual reports have been submitted.

Letter of Authorization granting the Alaska SeaLife Center authority as a volunteer under the Alaska Region Stranding Network, effective May 1, 1998.

**Other Permits Needed**

Not applicable

## *VII. References*


Acevedo, H.F. 2002. Human chorionic gonadotropin (hCG), the hormone of life and death: a review. J of Exp Therap and Onc 2:133

Adams, G.P., J.W. Testa, C.E.C. Goertz, R.R. Ream, and J.T. Sterling. *In press.* Ultrasonographic characterization of reproductive anatomy and early embryonic detection in the northern fur seal (Callorhinus ursinus) in the field.

Asa, C.S., and O.J. Ginther. 1982. Glucocorticoid suppression of oestrus, follicles, LH and ovulation in the mare. J Reprod Fertil. Suppl 32:247-51.

Challis, J.R.G., S.G. Matthews, W. Gibb, and S.J. Lye. 2000. Endocrine and paracrine regulation of birth at term and preterm. Endo. Rev. 21 (5): 514-550.

Chandra RK. 1991. Interactions between early nutrition and the immune system. In The Childhood Environment and Adult Disease. ed. GR Bock & J Whelan, pp77-88. Chichester: Wiley-Interscience.

Chandra RK. 2002. Nutrition and the immune system from birth to old age. Eur J Clin Nut. 56 (3): 573-576.

Cheng, S. J., and Z.Q. Zheng. 2004. Early Pregnancy Factor in Cervical Mucus of Pregnant Women. Am J Reprod Imm 51, 102-105

Cook, R.C., J.G. Cook, R.A. Garrott, L.L. Irwin, and S.L. Monfort. 2002. Effects of diet and body condition on fecal progestagen excretion in elk. J. Wildl. Dis. 38(3): 558-565.

Dloniak, S.M., J.A. French, N.J. Place, M.L. Weldele, S.E. Glickman, and K.E. Holekamp. 2004. Non-invasive monitoring of fecal androgens in spotted hyenas (*Crocuta crocuta*). Gen. Comp. Endo. 135: 51-61.

Gardiner, K.J., I.L. Boyd, B.K. Follett, P.A. Racey, and P.J.H. Reijnders. 1999. Changes in pituitary, ovarian, and testicular activity in harbour seals (*Phoca vitulina*) in relation to season and sexual maturity. Can. J. Zool. 77: 211-221.

Gee, C.M., H.D. Geissinger, and R.M. Liptrap. 1991. Morphometric and steroid hormone changes associated with experimental anovulatory follicles in the sow. Can. J Vet Res 55(3):206-11.

Greig, D.J., K. Mashburn, M. Rutishauser, F.M.D. Gulland, T. Willliams, and S. Atkinson. 2006. Seasonal changes in circulating progesterone and estrogen concentrations in the California sea lion (*Zalophus Californianus*) Journal of Mammalogy. In Press

Haddad J.J., N.E. Saade, and B. Safieh-Garabedian. 2002. Cytokines and neuro-immune-endocrine interactions: a role for the hypothalamic-pituitary-adrenal revolving axis. Journal of Neuroimmunology. Dec;133(1-2):1-19

Harmon, H, M. Castellini, J. Rowell, and S. Atkinson. 1999. Steller sea lion reproductive endocrinology and physiology. 13th Biennial Conf. Biol. Mar. Mammals

Holmes, E.E., L.W. Fritz, A.E. York, and K. Sweeny. *In review.* Fecundity declines in Steller sea lions suggest new conservation and research priorities.

Ishikawa, A., H. Sakamoto, S. Katagiri, and Y. Takahashi. 2003. Changes in sexual behavior and fecal steroid hormone concentrations during the breeding season in female Hokkaido brown bears (*Ursus arctos yesoensis*) under captive condition. J. Vet. Med. Sci. 65(1): 99-102.

Jacobs, R., I.R. Young, S.A. Hollingsworth, and G.D. Thorburn. 1994. Chronic administration of low doses of adrenocorticotropin to hypohysetomized fetal sheep leads to normal term labor. Endocrinol. 134:1389-1394.

Larkin, I.L.V., T.S. Gross, and R.L. Reep. 2005. Use of fecal testosterone concentrations to monitor male Florida manatee (*Trichechus manatus latirostris*) reproductive status. Aqua Mam. 31(1): 52-61.

Larson, S., C.J. Casson, and S.K. Wasser. 2003. Noninvasive reproductive steroid hormone estimates from fecal samples of captive female sea otters (*Enhydra lutris*). Gen Comp Endo. 134: 18-25.

Lee J.J. and A.P. Rubin. 1993. Breast feeding and anesthesia. Anesthesia 48:616-625.

Lesage, J., B. Blondeau, M. Grino, B. Breant, and J.P. Duouy. 2001. Maternal undernutrition during late gestation induces fetal overexposure to glucocorticoids and intrauterine growth retardation, and disturbs the hypothalamo-pituitary adrenal axis in the newborn rat. Endocrinol. 142(5)1692-1702.

Liptrap, R.M. 1993. Stress and reproduction in domestic animals. Ann N Y Acad Sci 697:275-84.

Litz B., K. Mashburn, L. Petrauskas, and S. Atkinson. 2005. Non-Invasive monitoring of testosterone in an endangered species, the Steller sea lion (*Eumetopias jubatus*). Poster presentation to the Marine Mammal Conference, San Diego, CA

Mashburn, K. L., and S. Atkinson. 2004. Evaluation of adrenal function in serum and feces of Steller sea lions (*Eumetopias jubatus*): influences of molt, gender, sample storage, and age on glucocorticoid metabolism. Gen. Comp. Endocrinol. 136(3):371-81.

Mashburn, K. L., and S. Atkinson. 2006. Seasonal and predator influences on adrenal function in adult Steller sea lions:Gender matters. Gen. Comp. Endo. doi:10.1016/j.ygcen.2006.08.009.

Myers, M J., L.D. Rea, and S. Atkinson. 2006. The Effects of Age, Season and Geographic Region on Thyroid Hormones in Steller Sea Lions (*Eumetopias jubatus*). Comparative Biochemistry and Physiology. Molecular & Integrative Physiology 145(1):90-98. 2006.

National Marine Fisheries Service (NMFS). 1992. Recovery plan for the Steller sea lion (*Eumetopias jubatus*). Prepared by the Steller Sea Lion Recovery Team for the National Marine Fisheries Service, Silver Spring, Maryland, 92p.

National Marine Fisheries Service (NMFS). 2006. Draft Revised Recovery Plan for the Steller sea lion (*Eumetopias jubatus*). National Marine Fisheries Service, Silver Spring, MD. 285 pages.

Petrauskas, L., P. Tuomi, and S. Atkinson. 2006. Noninvasive Monitoring of Stress Hormone Levels in a Female Steller Sea Lion (Eumetopias Jubatus) Pup Undergoing Rehabilitation. Journal of Zoo And Wildlife Medicine 37(1):75-78. 2006.

Petrauskas, L.R., and S.K. Atkinson. 2006. Variation of Fecal Corticosterone Concentrations in Captive Steller Sea Lions (Eumetopias Jubatus) in Relation to Season and Behavior. Aquatic Mammals 32(2):168-174. 2006.

Pietraszek J.R., and S. Atkinson. 1994. Concentrations of oestrone sulfate and progesterone in plasma and saliva, vaginal cytology, and bioelectric impedance during the oestrous cycle of the Hawaiian monk seal. Mar. Mamm. Sci. 10:430-441

Pickard A.R., T. Abaigar, D.I. Green, and W.V. Holt. 2001. Hormonal Characterization of the reproductive cycle and pregnancy in the female Mohor gazelle (*Gazella dama mhorr*). Reproduction. 122: 571-580

Pitcher K.W., and D.G. Calkins D.G. 1981. Reproductive biology of Steller sea lions in the Gulf of Alaska. J Mammol 63:720-737.

Pitcher K.W., D.G. Calkins, and G.W. Pendleton. 1998. Reproductive performance of female Steller sea lions: an energetics-based reproductive strategy? Can J of Zool 76:2075-2083.

Rolland, M.R., K.E. Hunt, S.D. Kraus, and S.K. Wasser. 2005. Assessing reproductive status of right whales (*Eubalaena glacialis*) using fecal hormone metabolites. Gen. Comp. Endo. 142: 308-317.

Schwarzenberger, F., E. Mostl, R. Palme, and E. Bamberg. 1996. Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. Anim. Repro. Sci. 42: 515-526.

Sun, M., M. Ramirez, J.R.G. Challis, and W. Gibb. 1996. Immunohistochemical localization of the glucocorticoid receptor in human fetal membranes and decidua at term and preterm delivery. J. Endocrinol. 149:243-248.

Sutton-Riley J.M., S.A. Khanlian, F.W. Byrn, and L.A. Cole. 2006. A single serum test for measuring early pregnancy outcome with high predictive value. Clin Biochem. Jul; 39(7):682-7

Thompson, K.V., K.L. Mashburn, and S.L. Monfort. 1998. Characterization of estrous cyclicity in the sable antelope (*Hippotragus niger*) through fecal progestagen monitoring. Gen. Comp. Endo. 112(1): 129-137.

Vighio, G.H., and R.M. Liptrap. 1990. Plasma hormone concentrations after administration of dexamethasone during the middle of the luteal phase in cows. Am J Vet Res 51(11):1711-4.

Wasser, S.K., S. Papageorge, C. Foley. and J.L. Brown. 1996. Excretory fate of estradiol and progesterone in the African elephant (*Loxodonta africana*) and patterns of fecal steroid concentrations throughout the estrous cycle. Gen. Comp. Endo. 102: 255-262.

Weber, KT. (2003) A neuroendocrine-immune interface. The immunostimulatory state of aldosteronism. Herz 28(8):692-701.

Whittle, W.L., F.A. Patel, N. Alfaidy, A.C. Holloway, M. Fraser, S. Gyomorey, S.J. Lye, W. Gibb, and J.R.G. Challis. 2001. Glucocorticoid regulation of human and ovine parturition: The relationship between fetal hypothalamic-pituitary-adrenal axis activation and intrauterine prostaglandin production. Biol. Reprod. 64, 1019-1032.

## *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 the regulations promulgated there under, as indicated in Section I of this application:*

*The Endangered Species Act of 1973 (16 U.S.C. 1531-1543) and regulations (50 CFR Part 222); and/or*

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

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

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

Tylan Schrock December 1, 2006
Executive Director

*Enc:*
*CV's- Brett Long, Justin Jenniges, Kendall Mashburn*
*MOU- ASLC, Mystic, Vancouver, Oregon Coast*
*Schematics- ODL and SSLH*
*USDA APHIS report 8.26.2006*
*Veterinary certification*

# Brett M. Long

P.O.Box 2613
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brett_long@alaskasealife.org

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## Professional Experience:

**Alaska SeaLife Center, Seward Alaska**
Mammal Curator July 2006-Present

- Responsible for directing and managing all aspects of the Alaska SeaLife Center mammal division including marine mammal husbandry of permanent resident, research, and rehabilitation animals.
- Plans and directs the acquisition, display, care, and maintenance of all mammal species.
- Supervises personnel matters within the Mammal Division. Sets performance standards for staff and prepares annual performance evaluations.
- Works in conjunction with the research department mangers to coordinate and facilitate mammal research sampling and data collection protocols and schedules.
- Works with department heads in maintaining exhibits and collections in a manner stimulating and factual to the visitors
- Provides advice to life support and other departments on design and operation of mammal habitats and rehabilitation areas.
- Serves as an animal care advisor specific to marine mammals on the ASLC Institutional Animal Care and Use Committee (IACUC).

**Joseph J. Long Marine Laboratory, University of California, Santa Cruz**
Marine Mammal Performance and Physiology Lab November 1999 – August 2006
*Research Training Coordinator & Project Manager*

- Coordinate and assist in animal training, animal handling, research goals and volunteer program.
- Ensure compliance with federal and state regulations for the holding and use of marine mammals in research. This includes oversight for National Marine Fisheries Service permits (NMFS), Chancellor's Animal Research Committee (CARC) Institutional Animal Care and Use Committee (IACUC), US Fish & Wildlife Service permits, and Animal and Plant Health Services (APHIS).
- Serve as liaison for marine mammal research collaborations within and outside of the University of California, Santa Cruz.
- Provide oversight for marine mammal facilities maintenance. Including but not limited to hands-on projects to the coordination of volunteers and UCSC employees for the maintenance of tanks and water quality.
- Serve as liaison with the UCSC veterinarian (Dr. Dave Casper) to monitor the health of the animals in the collection.
- Assist in the coordination of laboratory and field research projects involving marine mammals.
- Present regular reports of lab activities and animal status to the Principal Investigator
- Ensure quarantine regulations are met for new research animals and opportunistic stranded or rehabilitated animals. Includes making decisions regarding the placement of live-stranded marine mammals.
- Prepare and present an interactive teaching lecture series designed for current animal care and training staff.
- Direct the training of staff and volunteers on the project.

# Brett M. Long

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**Moss Landing Marine Laboratories, California State University, San Jose**
Vertebrate Ecology Laboratory January 2000-August 2006
*Research Technician*

- Participate in and coordinate field research teams to capture, tag and collect morphometric data on Pacific harbor seals along the California coastline and Channel Islands.

**Moss Landing Marine Laboratories, California State University, San Jose**
Small Boats Operations August 1998 – April 2001
*Marine Technician*

- Deployed all scientific equipment used for data collection.
- Performed in scientific diving for research projects.
- Captained scientific vessels on data collection cruises.
- Crewed aboard the R/V John H. Martin, a 56' research vessel.
- Performed maintenance of small boats designed for research.

**Moss Landing Marine Laboratory, California State University, San Jose**
S.L.E.W.T.H Project March 1995 – March 1999
*Senior Trainer & Marine Mammal Facility Manager*

- Participated in husbandry and training of 3.1 California sea lions (*Zalophus californianus*).
- Instructed training staff in proper application of operant and classical conditioning.
- Designed, constructed, and maintained enclosures, pools, support buildings, water systems and equipment needed for sea lion living quarters and data collection.

**Joseph J. Long Marine Laboratory, University of California, Santa Cruz**
Marine Mammal Performance and Physiology Lab June 1992 – March 1997
*Senior Trainer*

- Trained 2.0 Atlantic bottlenose dolphins (*Tursiops truncatus*), 1.1 California sea lions (*Zalophus californianus*) and 1.1 Southern sea otters (*Enhydra lutris*).
- Supervised volunteer training and animal care staff.
- Performed animal husbandry tasks, such as preparing food and vitamins, cleaning pools, maintaining filtration systems and testing water for temperature, chlorine, pH and coliform levels.
- Facility maintenance.

## Education:

University of California Concurrent Enrollment, Santa Cruz April 1996-December 1996
Marine biology Emphasis

Cabrillo Community College August 1991 – December 1992, August 1994 – December 1995
Biology Emphasis

Chaffey Community College September 1989 – June 1991, January 1993 – June 1994
Biology Emphasis

# Brett M. Long

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## Professional Affiliation:

International Marine Animal Trainer's Association (IMATA) – Active professional member

## Current Certifications:

- National Association of Underwater Instructors- Instructor
- Scientific Diver- University of California, Santa Cruz
- CPR and First Aid Instructor
- Diver alert Network (DAN) $O_2$ Provider
- California Department of Boating and Waterways-Certificate of Completion
- Oiled Wildlife Care Network (OWCN) supervisor, University of California, Davis

## Presentations:

Fink, Traci L., Gafney, Jen, and **Long, B**. *Training Challenges On A Research Project Studying The Metabolic Costs Associated With Reproduction In California Sea Lions*, International Marine Animal Training Association, Kolmarden, Sweden. 10/04

Ballman, S, Dye, G, and **Long, B**. *Collaborating for Conservation*, International Marine Animal Training Association, Long Beach, CA, 11/03

Gafney, J and **Long, B**. Repetitive *Blood Draws With No Primary Reinforcement*, International Marine Animal Trainers Association, Orlando, FL. 11/02

Long, B. *When Bad Otters Go Good: Training Rehabilitated Sea Otters*, Alaska Sea Life Center, Seward, AK. 3/02

Long, B. *Marine Mammal Performance and Physiology Project* 2002, 2004, 2005, and 2006, Seymour Marine Discovery Center, Long Marine Laboratory, Santa Cruz, CA. 2/04

## Publications:

Williams, T., McDonald, K., Rutishauser, M., **Long, B.**, Gafney, J., Hurley, W., Aiello, K., Richter, B. *Expending Energy By The Step Or Stroke In Elite Runners and Swimmers*. (manuscript submitted)

Arnold, C.M., Jessup, D.A., Casper, D., Murray, M.J., **Long B.M.**, Gafney, J., Fink, T.L. and Williams, T.M. *A Program to Protect Captive Southern Sea Otters (Enhydra lutris nereis) from Morbillivirus Exposure at a Veterinary Care and Research Facility*. (Submitted to the Wildlife Disease Association (WDA) for presentation at the WDA conference July 28 – August 1 2002.

# Justin Jenniges

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(907) 224-6329
Justin_Jenniges@alaskasealife.org

## Professional Experience

**Field Research Coordinator** **Alaska SeaLife Center** **01/06 – Present**

- Coordinate research objectives among departments
- Perform boat based support
- Capture juvenile Steller sea lions and personally branded 11
- Collect biological samples
- Write permit applications and annual reports

**Research Support Technician** **Alaska SeaLife Center** **02/05 – 12/05**

- Assisted Field Research Coordinator
- Research, design, and fabricate specialized equipment
- Personally branded 12 Steller sea lion pups and 3 juveniles
- Perform boat based support

**Visiting Scientist** **Oregon Department of Fish and Wildlife** **07/05 – Present**

- Captured 200 Steller sea lion pups and personally branded >20
- Captured 50 adult California sea lions and personally branded 10
- Collected biological samples, then attached scientific instrumentation
- Trained colleagues to use remote controlled video cameras

**Research Technician** **Pacific States Marine Fisheries Commission** **03/02 – 10/04**

- Used remote controlled video cameras for pinniped mark recapture studies
- Captured and sampled over 2000 sea lions and personally branded >300
- Lead monthly pinniped surveys
- Used floating trap to capture and brand large adult California sea lions
- Captured approximately 300 Northern fur seal pups annually
- Captured and instrumented Pacific harbor seals
- Operated and maintained 23' Boston Whaler

## Education

**M.A. Ecology** **St. Cloud State University** **12/01**

- "Age Determination of Common Snapping Turtles in West Central Minnesota"
- Presented at annual meeting of The Wildlife Society- Minnesota Chapter

**Specialized Certificate: GIS** **St. Cloud State University** **07/00**

- Equivalent to a Geographic Information Systems minor

**B.S. Biology–Zoology** **St. Cloud State University** **08/98**

**Kendall Mashburn**
**P.O. Box 835, 1120 4th Ave #4**
**Seward, Alaska 99664**
**(907)224.4707**

**Education:**

| | | | |
|---|---|---|---|
| 1988 – 1990 B.S. Animal Science | Cornell University | Ithaca, NY. | Reproduction/Embryology |
| 1986 – 1988 | Cumberland College | Williamsburg, KY. | Biology /Genetics/Embryology |

**Professional Experience:**

| | |
|---|---|
| September 2006 – Present | Peer Reviewer: General and Comparative Endocrinology.<br>Elsevier |
| April 2001 – Present | University of Alaska, Fairbanks Alaska SeaLife Center.<br>Seward, Alaska<br>Science Program Coodinator |
| October 1991 – March 2001. | Smithsonian Institution / National Zoological Park /<br>Conservation and Research Center. Front Royal, VA.<br>Endocrine Research Biotechnician. |
| June 1990 - December 1990. | New York State College Of Veterinary Medicine, Cornell University.<br>Dept. of Clinical Sciences and Medicine/ Dept. of Physiology.<br>Research Technician / Animal Handler. |
| December 1989 - June 1990. | New York State College of Veterinary Medicine, Cornell University.<br>Dept. Animal Science/Dept. of Physiology<br>Technician / Undergraduate Research |
| September 1987 - May 1988. | Cumberland College. Williamsburg, KY<br>Dept. of Biology<br>Teaching Assistant / Biology Tutor |
| May 1987 - September 1987. | Corbin Animal Clinic. Corbin, KY.<br>Veterinary Technician |

**Technical Experience:**

High performance liquid chromatography; RIA ($^{125}I$, $^{3}H$, $^{14}C$; steroids, proteins) including validations (parallelism. accuracy, HPLC, preparation of samples for mass spec, physiological validations/challenges/radiolabel infusions); RIA and EIA (all phases including enzyme conjugation) development and modification to accommodate a wide variety of exotic species; iodination of protein hormones; conjugation of steroid hormones; standard and QC preparation; extraction of reproductive steroids from blood, feces and urine; pituitary removal and purification of pituitary hormones; semen collection, electroejaculation and AI protocols in several domestic and exotic species; semen evaluation, cryopreservation; reproductive tract removal/embryo and oocyte flushing/follicle evaluations and manipulation; CIDR preparation, modification and insertion; development of estrus synchrony protocols in exotic hoofstock; photo and light microscopy; spectrophotometry; hemocytometer use; sample collection (blood, urine, and feces) from exotic and domestic animals; vaginal smears from exotic and domestic animals; necropsy of both exotic and domestic species; administration of immunizations, medications and anesthesia in exotic and domestic animals; calculation, measurement and processing of medications and treatments; CIDR preparation, modification and insertion; assisting and record keeping during surgical procedure; animal manipulation based on experimental protocols (mammals and birds); computer analysis of data (IBM and Macintosh systems), graphic representation and interpretation of results; independent decisions regarding field studies and equipment based on prevailing conditions in foreign countries; experimental design, implementation and write-up for publication; protocol modification and training in developing countries; mobile lab transport overseas and domestically; ensuring passage of equipment through customs; maintenance of equipment and electrical modifications in the field both at sea and on land in remote locations.

**Managerial Experience:**

Complete oversight and management of an endocrine research facility for both a Science Director and research veterinarian; safety officer for entire laboratory facility for the Conservation and Research Center; independent decisions regarding the implementation of experimental protocols based on prior data; scheduling priorities for sample collection, processing, and analysis; maintenance of records (samples, treatments, behavioral observations, medical applications) from several different species and studies occurring simultaneously; oversight of grant account spending; ordering and maintenance of equipment/supplies; maintenance of correspondence records between study collaborators and investigators; maintenance of material safety data records, safety equipment, and safety protocols for research facility; maintenance of $^{125}I$ isotope records in accordance with NRC guidelines; shipment and follow-up of research equipment and materials to domestic and overseas locations in accordance with state, federal, and international guidelines and regulations; international and domestic permit application and renewal for both CITES and non-endangered species; logistics planning and implementation for international travel as a Smithsonian and University representative, public presentation of my own current research as well as that of my supervisor and graduate students; writing proposals; procedure and drug use justification in endangered species to satisfy permit, IACUC, and public concerns and requirements; sub-contract awards justifications and ensuring mutual acceptance of sub-contracts between disparate institutions.

**Peer Reviewed Publications:**

**Mashburn, K.L.** and Atkinson, S.A. (2006). Seasonal variation in reproductive steroid response to acute stress in adult Steller sea lions. In Preparation.

**Mashburn, K.L.** and Atkinson, S.A. (2006). Variability in leptin and adrenal response in juvenile Steller sea lions to ACTH in different seasons. In Review: *Gen. Comp. Endocrinol*.

Greig, D. J., **Mashburn, K**., Rutishauser, M, Gulland, F., Williams, T., Atkinson, S. (2006). Seasonal changes in circulating progesterone and estrogen concentrations in the California sea lion (*Zalophus californianus*) In Press: J. Mammol.

**Mashburn, K.L.,** and Atkinson, S.A (2006). Seasonal and predator influences on adrenal function in adult Steller sea lions: Gender matters. *Gen Comp. Endocrinol*. doi:10.1016/j.ygcen.2006.08.009.

Richmond, J.P., Burns, J.M., Rea, L.D**., Mashburn, K.L.** (2005). Postnatal ontogeny and hematology in free-ranging Steller sea lions (*Eumetopias jubatus*). *Gen. Comp. Endocrinol*. 141:240-247.

**Mashburn, K.L**. and Atkinson, S. (2004) Evaluation of adrenal function in serum and feces of Steller sea lions (*Eumetopias jubatus*): influences of molt, gender, sample storage, and age on glucocorticoid metabolism. *Gen. Comp. Endocrinol*. 136:371-381.

Hosack, D.A., Monfort, S.L., Miller, K.V., **Mashburn, K.L**., Ware, L., and Marchinton, R.L. (1999) Stag exposure advances LH surge and behavioral estrus in Eld's deer (*Cervus eldi thamin*) hinds after CIDR device synchronization of estrus. *Theriogenology* 51(7):133-142.

Garrot, R.A., Monfort, S.L., White, P.J., **Mashburn, K.L**., and Cook, J.G. (1998) One sample pregnancy diagnosis in elk using fecal steroid metabolites. *J. Wildl. Dis.* 34(1):126-131.

Thompson, K.V., **Mashburn, K.L**., Monfort, S.L. (1998) Characterization of estrus cyclicity in the sable antelope (*Hippotragus niger*) through fecal progestagen monitoring. *Gen. Comp. Endocrinol*. 112:129-137.

Monfort, S.L., **Mashburn, K.L.,** Brewer, B.A. and Creel, S.R. (1998) Evaluating adrenal activity in African wild dogs (*Lycaon pictus*). *J. Zoo. Wildl. Med.* 29(2):129-133.

Monfort, S.L., Creel, S., **Mashburn, K**, Wasser, S.K. (1997) Steroid metabolism and validation of noninvasive endocrine monitoring in the African wild dog (*Lycaon pictus*).
*Zoo Biol*. 16:533-548.

# MEMORANDUM OF UNDERSTANDING

**Between**

**SEWARD ASSOCIATION FOR THE ADVANCEMENT OF MARINE SCIENCE (SAAMS)** dba
**ALASKA SEALIFE CENTER (ASLC)**
**SEWARD, ALASKA**
**And**
**SEA RESEARCH FOUNDATION, INCORPORATED (SRF)**
**Mystic, Connecticut**

**WHEREAS,** ASLC and SRF have similar missions in marine scientific research and education and have common goals to promote ocean and marine sciences in the U.S.

**WHEREAS,** ASLC and SRF agree to cooperate through productive scientific research and education. Both Parties are major, independent, nonprofit organizations in the United States of America, located near marine environments, share a common interest in maintaining marine biodiversity, are renowned for their research accomplishments, and operate world-class public education venues. Both institutions share a common bond for the conservation and enhancement of U.S. marine resources including its endangered and threatened species. The parties agree to collaborate in progressing research and other areas of mutual interest as described.

**WHEREAS,** ASLC and SRF agree that mutual cooperation will benefit not only, the states of Alaska and Connecticut, but the entire United States, through education and research for improving the health and the sustainable use of United States marine ecosystems and natural resources.

**NOW, THEREFORE,** it is agreed by both Parties that the purpose of this Memorandum of Understanding is to facilitate cooperation and support scientific research and educational proposals. Alternating between the institutions, at least one representative from each of the functional areas of research, education, public programs and institution management will meet at least once a year. It is expected that members representing these functional areas will meet and interact as necessary to carry out this cooperative mission. Each institution will assign an administrative contact to coordinate meetings, keep records, and facilitate interactions.

**FURTHERMORE,** it is agreed by both Parties that efforts will be made in the following areas of mutual, collaborative interest: aquatic animal health research including veterinary medicine, nutrition, immunology, infectious disease, reproduction, endocrinology, strandings, stress and environmental impacts on the immune system; immersion theater programs; K-12 education; public exhibits; marine exploration; marine biomedicine and biotechnology; education; marketing; communications; United States legislative issues; volunteer management; fund raising; public visitorship and any other areas that shall be deemed by both Parties to be of mutual interest. A separate cooperative agreement will be drawn for specific individual projects or programs.

This agreement by both Parties will also make specific efforts in collaborative areas of interest including areas of common research and educational interests with colleagues from each institution:

1. Methods of equitable allocation of the time and costs for the use of high-cost research equipment, facilities, libraries, ships and underwater vehicles.
2. Ways to effectively implement and promote graduate and undergraduate educational programs, as well as K-12 programs.
3. Research focusing on health related issues of Steller sea lions, northern fur seals, and other aquatic species.
4. Joint participation in one another's membership activities, such as lectures, courses and trips.
5. Use of each institution's conference centers and classrooms for large meetings, workshops and educational programs.

**BOTH PARTIES HEREBY** reaffirm their existing commitment to conduct good faith efforts to complement, enhance, expand and constructively use the available resources and services for the benefit of the scientific community and the general public.

**IN WITNESS WHEREOF** Officers of Alaska SeaLife Center and Sea Research Foundation, Incorporated affix their signatures this 17th day of March, 2006, to consummate this agreement.

For:
**Seward Association for the Advancement of Marine Science,** dba
**Alaska SeaLife Center**
P.O. Box 1329
301 Railway Avenue
Seward, AK 99664

3/23/06
**Tylan Schrock** Date
Executive Director

3/17/06
**Robert Spies** Date
President, Board of Directors

For:
**Sea Research Foundation, Incorporated**
55 Coogan Boulevard
Mystic, CT 06355

3/30/06
**Gerard N. Burrow, M.D.** Date
President & CEO

4/11/06
**George M. Milne, Jr. Ph.D.** Date
Chairman, Board of Trustees

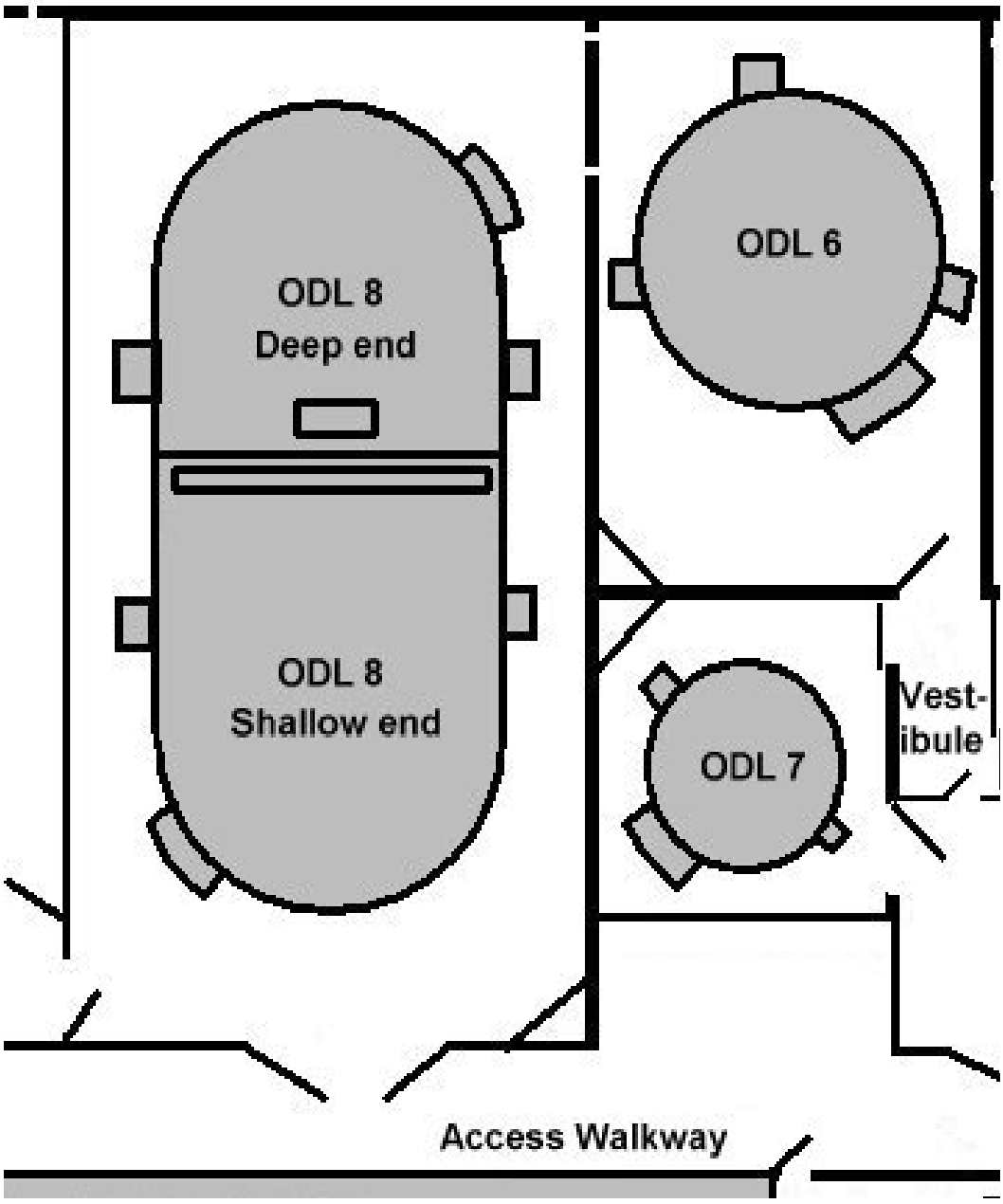



**Figure 1. ASLC outdoor lab (ODL)**

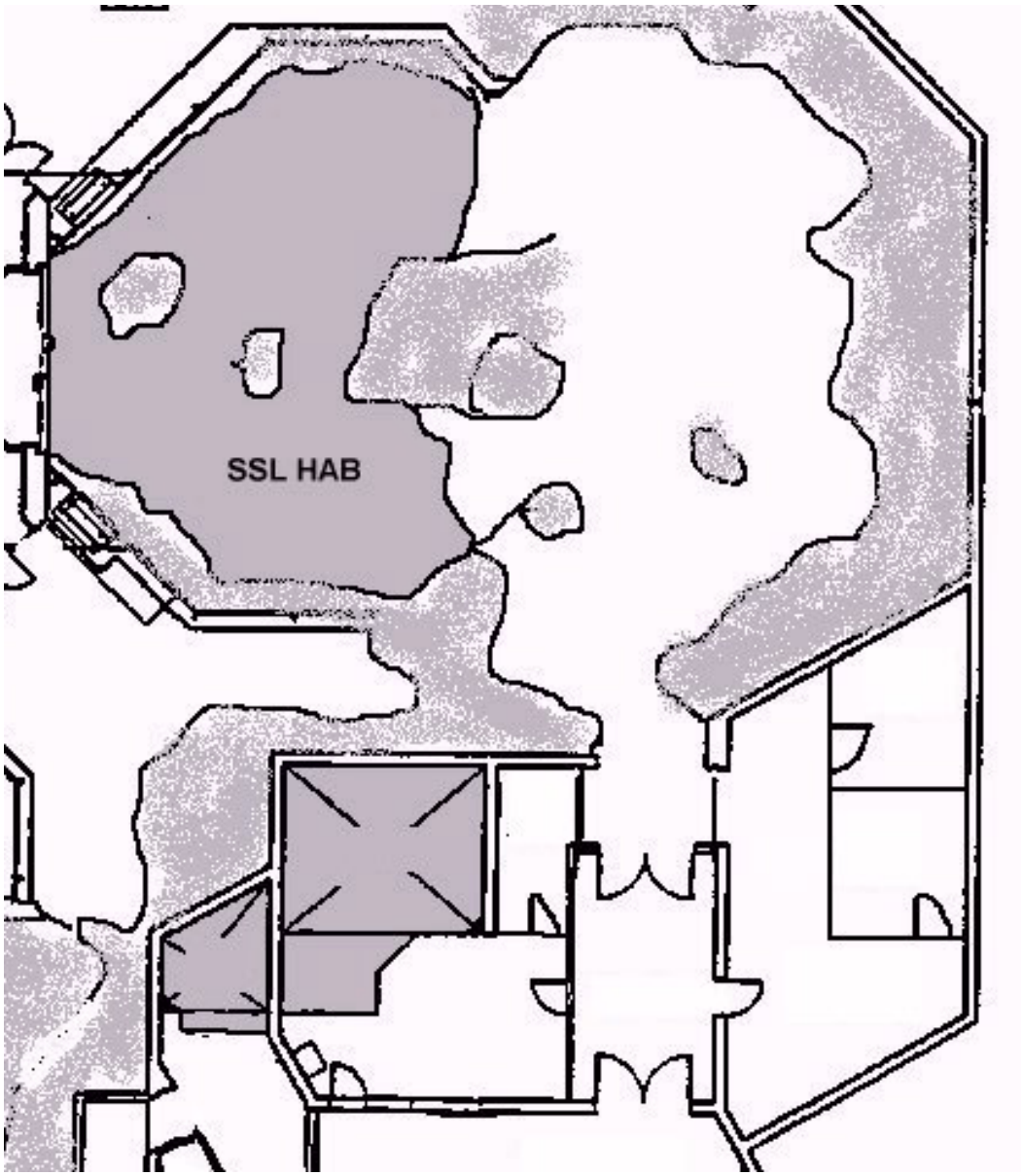



**Figure 2. ASLC Steller sea lion habitat (SSLH)**

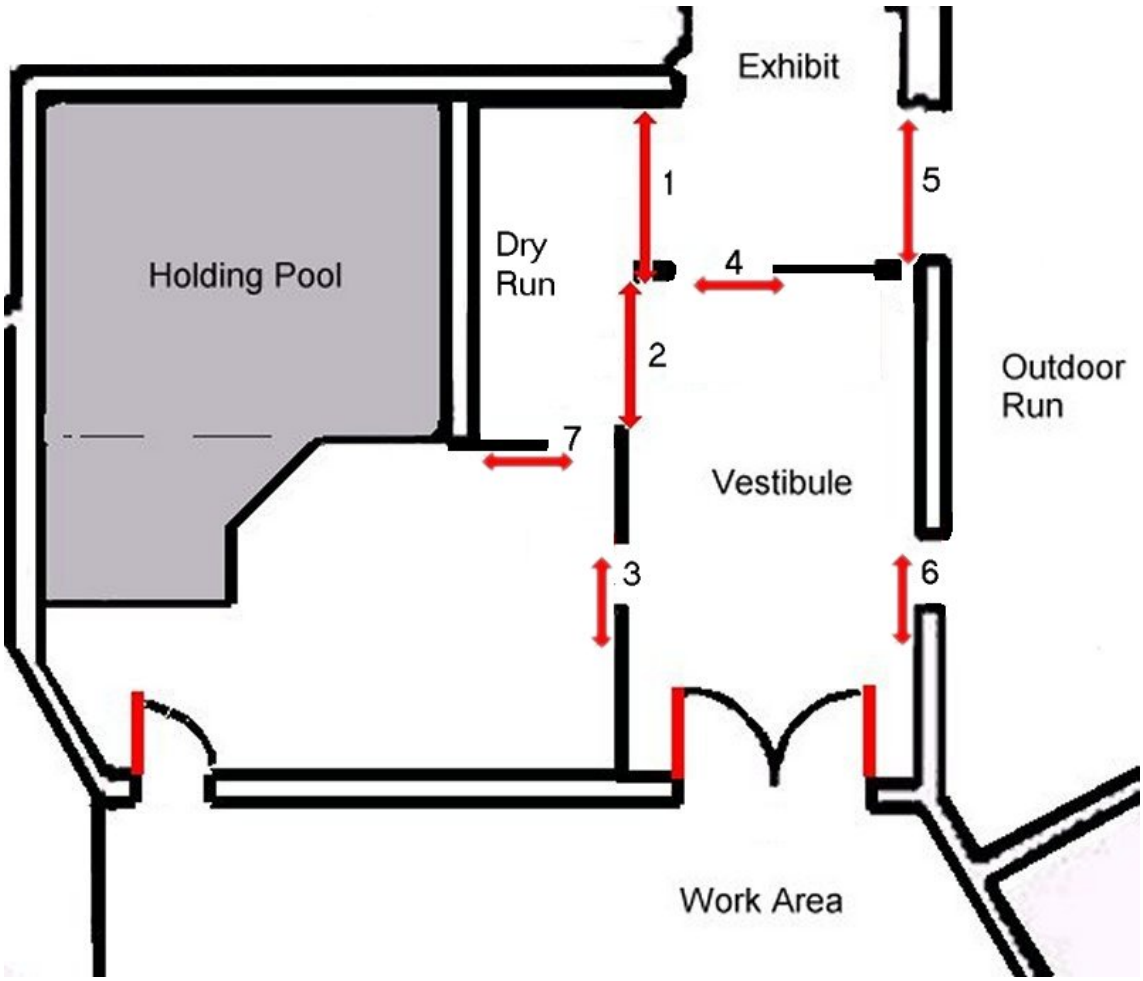
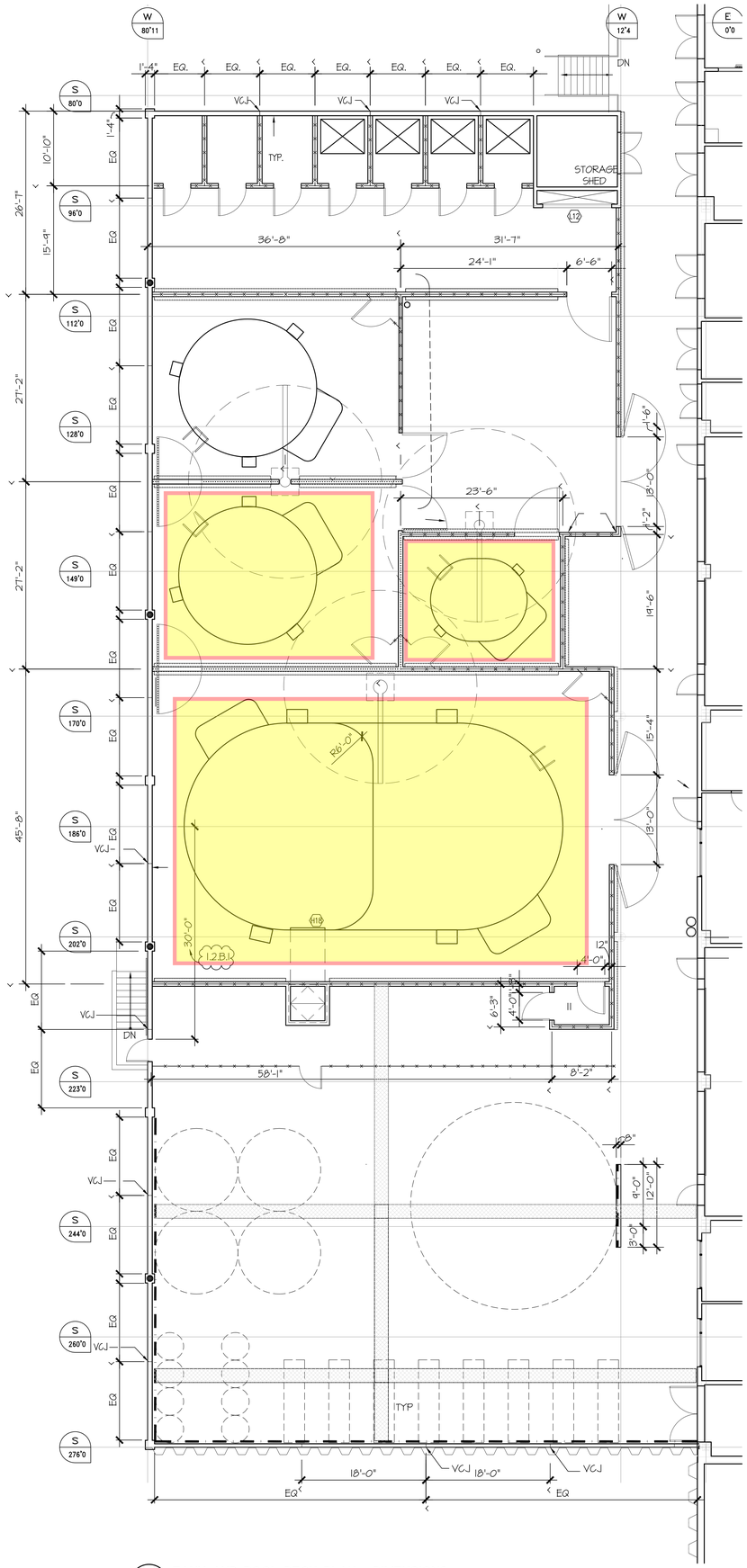
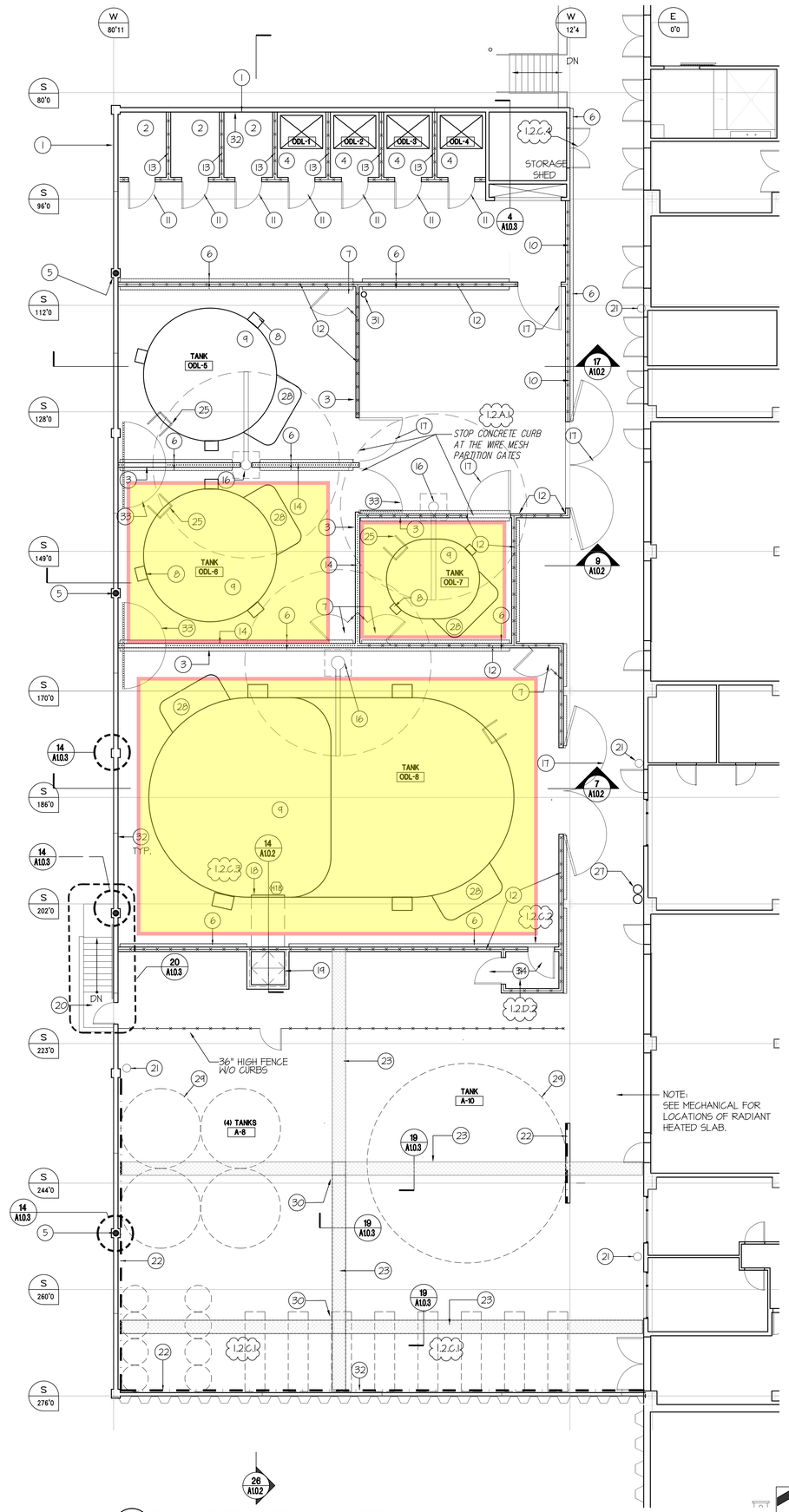



**Figure 3. ASLC Steller sea lion habitat (SSLH) close up.**




1 A1.0.1 TANK AND POOL DECK PLAN - DIMENSIONS

APPROXIMATE SCALE: _"=1'-0"




2 A1.0.1 TANK AND POOL DECK PLAN - NOTES

APPROXIMATE SCALE: _"=1'-0"

NORTH

## KEYNOTES

1. 10'-0" HIGH CAST-IN-PLACE CONCRETE WALL.
2. MARINE MAMMAL RESEARCH PENS.
3. 6" CONCRETE CURB
4. MARINE MAMMAL RESEARCH POOLS.
5. LIGHT POLE ON CONCRETE BASE. SEE ELECTRICAL
6. TRENCH DRAIN
7. (1.2.E.1) UTILITY CLOSET W/ FRESH AND SALT WATER SUPPLY, AND ELECTRICAL OUTLET. *SEE DETAIL 2/A1.0.3 AND DETAIL 3/A1.0.3*
8. (1.2.F.1) *SKIMMERS. SEE STRUCTURAL AND LIFE SUPPORT DRAWINGS FOR SIZE AND CONFIGURATION*
9. CAST-IN-PLACE CONCRETE POOL, TYPICAL.
10. *8'-0" HIGH WIRE MESH PARTITION FENCE ON 2'-0" HIGH CONCRETE CURB.*
11. (1.2.A.1) *4'-0" WIDE x 6'-6" HIGH WIRE MESH PARTITION GATE*
12. *9'-6" HIGH WIRE MESH PARTITION FENCE ON 6" CONCRETE CURB.*
13. *6'-0" HIGH WIRE MESH PARTITION FENCE ON 6" CONCRETE CURB.*
14. *9'-6" HIGH HEAVY DUTY STEEL BAR FENCING ON 6" HIGH CONCRETE CURB.*
15. EPOXY COATING ON VERTICAL AND HORIZONTAL SUURFACES
16. JIB CRANE.
17. (1.2.G.1) *6'-6" WIDE (EACH LEAF) BY 9'-6" FOOT HIGH GATE WITH CONTINUOUS HEADER BAR ABOVE.*
18. (1.2.H.1) ACRYLIC VIEW PANEL. *SEE SHEET A9.3.0 AND SPECIFICATIONS.*
19. UNDERWATER VIEWING AREA.
20. CAST-IN-PLACE CONCRETE STAIRS.
21. POTABLE WATER FOR WASH/DOWN CLEANING.
22. LIFE SUPPORT SYSTEM FRESH AND SALT WATER SUPPLY LOCATIONS. SEE LIFE SUPPORT
23. 24" WIDE TRENCH DRAINS.
24. UTILITY TRENCH. SEE LIFE SUPPORT
25. REMOVEABLE LADDER: INSTALL DECK SLEEVES PER MANUFACTURER'S RECOMMENDATIONS.
26. (1.2.A.1) *3'-0" HIGH WIRE MESH PARTITION FENCE. COORDINATE LOCATION OF FOUR GATES WITH ARCHITECT.*
27. PROPANE CYLINDERS W/ WALL MOUNTED RESTRAINTS.
28. WET HAUL OUT. SEE B/A1.0.3
29. PREFABRICATED TANKS FURNISHED AND INSTALLED BY OTHERS
30. (1.2.I.1) *36" X 36" X 48" DEEP CONCRETE SUMP WITH ALUMINUM GRATE.*
31. FRESHWATER SUPPLY HYDRANT
32. INSTALL INSULATION TYPE 6 W/ PROTECTION BOARD ALONG NORTH, WEST, AND SOUTH, SIDES OF THE TANK AND POOL DECK FROM BOTTOM OF DECK SLAB TO TOP OF FOOTING.
33. (1.2.J.1) *6'-6" WIDE X 9'-6" HIGH STEEL BAR GATE WITH CONTINUOUS HEADER BAR.*
34. (1.2.D.1) *4'-0" WIDE X 8'-0" HIGH WIRE MESH PARTITION GATE.*

**NOTE - REFER TO SPECIFICATIONS SECTION 01030 FOR INFORMATION ON DEDUCTIVE ALTERNATES WHICH MAY IMPACT THE WORK SHOWN ON THIS SHEET.**

## GENERAL NOTES

1. KEY NOTES REFER TO INFORMATION ON THIS SHEET ONLY.
2. SEE ELECTRICAL FOR OUTLETS ALONG GRID E/0'0.
3. INSTALL INSULATION TYPE 5 UNDER ALL AREAS OF RADIANT HEATED CONCRETE SLAB. INSULATION SHALL EXTEND 1'-0" BEYOND THE AREAS OF RADIANT HEAT SYSTEM WHERE POSSIBLE.
4. ALL HORIZONTAL CONCRETE WALKING SURFACES TO HAVE MEDIUM BROOM FINISH. TYPICAL
5. (1.2.K.1) *ALL CONCRETE TANKS, CURBS, AND HORIZONTAL WALKING SURFACES INCLUDING CAST TRENCH DRAINS AND ALL CONCRETE SURFACES OF SKIMMERS AND MAIN DRAINS TO HAVE TYPE 3 WATERPROOFING FINISH.*

Seward Association for the Advancement of Marine Science

# ALASKA SEALIFE CENTER

SEWARD, ALASKA

**NOT FOR CONSTRUCTION**

**NOT AS-BUILTS**


LIVINGSTON SLONE
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**DRAWING INCORPORATES ADDENDUMS 1 THOUGH 5**



**NOT CONTRACT DOCUMENTS**

**PROJECT NO.** 401.00
**DRAWN BY:** AMP/MP
**REVIEWED BY:** JB
**DATE** JAN. 31, 1996

**TITLE**

**OUTDOOR TANK AND POOL PLANS**

**SHEET NO.**

**A1.0.1**

United States Department of Agriculture
Animal and Plant Health Inspection Service

# INSPECTION REPORT

ELYONS
1693 cust_id
157311 insp_id
410 site_id

**S.A.A.M.S.**
**ALASKA SEALIFECENTER**

**P. O. BOX 1329**
**SEWARD, AK 99664**

**Customer ID:** 1693
**Certificate:** 96-R-0005
**Site:** 001
ALASKA SEALIFECENTER
**Inspection Type:** ROUTINE INSPECTION
**Date:** AUG-25-2006

No noncompliant items noted during this inspection.

End of report.

**Prepared By:** Elizabeth C. Lyons DVM, MS
ELIZABETH LYONS, V.M.O. , USDA, APHIS, Animal Care
**Title:** VETERINARY MEDICAL OFFICER , Inspector ID: 5009
**Date:** AUG-26-2006

**Received By:**
**Title:** Husbandry Director
**Date:** AUG-26-2006

Alaska SeaLife Center®
windows to the sea

16 November 2006

To Whom It May Concern:

I hereby certify that I am a qualified veterinarian licensed by the State of Alaska, and I am employed as the Senior Veterinarian at the Alaska SeaLife Center in Seward, Alaska, to oversee the care, maintenance and transport of animals at this facility.

As the attending veterinarian and a member of the ASLC IACUC, I have inspected the facilities and methods for both marine mammal care and maintenance and for transport of marine mammals. I also certify that they are adequate to ensure the well-being of the Steller Sea Lions specified in this permit application. These facilities and methods meet or exceed all applicable care and transport standards established under the Animal Welfare Act.

Sincerely,

Pam Tuomi, D.V.M.

301 Railway Avenue • P.O. Box 1329 • Seward, Alaska 99664
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