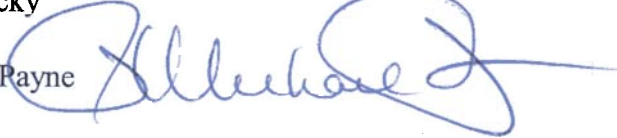




UNITED STATES DEPARTMENT OF COMMERCE  
National Oceanic and Atmospheric Administration  
NATIONAL MARINE FISHERIES SERVICE  
Silver Spring, MD 20910

JUL 16 2007

MEMORANDUM FOR: F/PR – James H. Lecky

FROM: F/PR1 – P. Michael Payne 

SUBJECT: Report on Application for an Amendment to Scientific Research Permit No. 881-1745-01 issued to the Alaska SeaLife Center (ASLC) for research on Steller sea lions: Recommendation for Issuance

This memorandum summarizes processing and decision recommendations for one pending application submitted for a permit to breed and conduct research on Steller sea lions (*Eumetopias jubatus*) in captivity. This application was processed concurrently with 12 other applications for research on Steller sea lions and northern fur seals (*Callorhinus ursinus*) including a batched Notice of Receipt in the *Federal Register*, and a batched decision memorandum signed on June 18, 2007 (attached), which contains all public and agency comments [including those from the Marine Mammal Commission, Humane Society of the United States, and National Marine Fisheries (NMFS) Service Alaska Region and Science Center] on this application; applicant and NMFS responses; and addresses National Environmental Policy Act, Marine Mammal Protection Act, Fur Seal Act, and Endangered Species Act requirements. This memorandum is an addendum to the original memorandum of decision signed on June 18, 2007, and summarizes outstanding issues related to facility space requirements.

The ASLC (Principal Investigator: Dr. Shannon Atkinson) requested a 5-year amendment to Permit No. 881-1745 to breed captive Steller sea lions at the ASLC, to produce up to four pups, and conduct studies related to gestation, lactation, and pup growth and development. Permit No. 881-1745, issued March 16, 2006 (59 FR 15387), currently allows studies on three adult (one male, two female) captive Steller sea lions held by the ASLC to investigate stress responses, endocrine and immune system function, and seasonal variations in normal biological parameters such as mass and body composition, and conduct of 'research and development' of external tags and attachments for future deployment on free-ranging animals. The purpose of the proposed amendment is to assess physical, metabolic, hormonal, and immunological changes related to gestation, lactation, and pup growth and development. Offspring produced would be held at the ASLC for long-term physiological studies. During gestation the adult animals would be subject to currently permitted sampling procedures, with additional study-specific testing on the samples themselves. Milk samples would be collected from adult females. Offspring produced would be subject to sedation, anesthesia, physical restraint, morphometric measurements, metabolic measurements, collection of urine and feces, blood sampling, and audio and visual recordings (e.g., audio, photographic, video, digital, thermal, radiographic). Offspring would be trained to encourage voluntarily participation in research activities to minimize the use of physical restraint, sedatives, or anesthetics during sampling. The ASLC requests one research-related mortality of any live-born Steller sea lion during the proposed study. The ASLC proposes that stillborn or spontaneously aborted pups not be considered related to the study or counted against any mortality allowance in their permit.



### Chronology

December 4, 2006	Date of application
February 15, 2007	Application published in <i>Federal Register</i> (72 FR 7420)
February 15, 2007	Application distributed
March 21, 2007	Extension of comment period published in <i>Federal Register</i> (72 FR 13255)
April 30, 2007	Close of public comment period
May 1, 2007	The Humane Society of the United States comments received
May 1, 2007	USDA Animal & Plant Health Inspection Service (APHIS) comments received
May 2, 2007	Marine Mammal Commission comments received
May 4, 2007	Comments forwarded to applicant for response
May 17, 2007	Applicant responses to comments due
May 21, 2007	Applicant response to reviewer comments received
May 31, 2007	Additional information requested
June 8, 2007	Applicant response received
June 27, 2007	Additional APHIS comments received

NMFS originally denied issuance of this permit in the June 18, 2007 memorandum because at that time, the USDA APHIS could not determine, based on the information in the application and additional information supplied by the applicant, whether the ASLC had adequate space to house additional Steller sea lions as might result from the proposed captive breeding. Since that time, NMFS provided APHIS with additional information from ASLC about its facilities and consulted with APHIS regarding whether there is space sufficient to conduct the proposed breeding activities. A final response from APHIS was pending at the time of signing the June 18, 2006 memo. At that time, PR1 did not recommend issuance of this permit until APHIS verification was received. On June 27, 2007, APHIS commented that: "In calculating available space based on information provided by ALSC and where they intend to place animals, their proposed scheme in the spreadsheet attached provides adequate space for all but where they propose to place pup 1. SSL holding is not acceptable for a male pup. In addition, the pups should be kept with the mother until weaning. This would be OK for ODL 5, 6, and 8. SSL Holding can only be used for mother/pup pair if the pup is female. It should be noted that to comply with AWA regulations and standards section 1.09 (separation), animals should be housed with conspecifics, and not alone."

The permit would authorize the ASLC to have up to 4 pups (not to exceed 3 males) born at the ASLC.

In addition, any stillborn or aborted animals resulting from captive breeding will count toward the authorized mortality (one Steller sea lion) under this amendment, as this would be directly related to the breeding authorized in this amendment. Hormone stimulation studies will not be permitted on lactating animals as the increased stress from the handling and repeated sampling of the mothers and the effects of these drug administrations relative to transfer to pup via milk were not fully considered in the environmental impact statement. In the ASLC's original application, the ASLC requested the ability to move Steller sea lions to and/or from the ASLC and other facilities for breeding management purposes. The original ASLC application did not specify animal ID's, numbers of animals, etc., that would be moved and indicated that this information would be provided at the time of such animal movements in a minor amendment request. PR1 indicated to the ASLC that this would not be processed as a minor amendment and requested details on any animal movements among facilities. The ASLC responded (see attached additional information) and requested that PR1 remove any animal transfer/import/export from the application.

### **Recommendation**

The applicant asserts that their research will further a *bona fide* scientific purpose consistent with the purposes of the MMPA, ESA, and applicable regulations, and that the methods of taking would be humane. The applicant asserts that the results of the research will directly benefit the species or stock, or otherwise fulfill a critically important research need. PR1 believes the research authorized by this permit would not likely have a significant adverse impact on marine mammal species or stocks. For these reasons, I recommend that you sign the permit.

**6/5/07 ASLC response to NMFS request for clarification and response to RE: ASLC response to public comments received on File No. 881-1745 (Alaska SeaLife Center, Steller sea lion captive research)**

Areas of concern:

1. Animal welfare:

a. Space issues-

The Alaska SeaLife Center (ASLC) is a USDA/APHIS licensed and inspected facility appropriate for the care and housing of marine mammals, including endangered Steller sea lions. The ASLC was designed to comfortably house many more sea lions than currently reside therein, and can easily house the additional animals requested in our amendment application. Currently, we have 13 pools that can each legally accommodate multiple animals. Please refer to the attached table and schematic diagrams for pool and habitat details.

**Comment [NMFS1]:** APHIS space requirements are based on adult size, no matter the age of an animal.

b. Injury caused by conspecifics-

The ASLC captives are currently housed together within the Steller sea lion habitat or the outdoor laboratory pools. Each of these enclosures has been designed to hold animals during breeding scenarios, and allow for exclusion of adult males from areas of refuge provided for females. For example, sliding doors can be secured in a partially opened state which allows adult females to pass through while excluding adult males.

**Comment [JJJ2R1]:** Our table had been prepared with the understanding that with the exception of sexually mature males, APHIS regulations indicate Steller sea lions can be managed as Group 1 pinnipeds meaning they can be kept in the same enclosure without separate dry holding areas. When more than one sexually mature male is present in a single habitat, APHIS regulations treat them as Group 2 pinnipeds which requires that they (the males) have distinct dry resting areas separated by visual barriers (Title 9 Part 3.104 d) (2) ii. In the current configuration, the ASLC can house 5 sexually mature male Steller sea lions simultaneously. ODL 8 is large enough that it could be modified with visual barriers (e.g. rocks, fences, foliage, etc) to allow additional males to reside. The attached table has been modified to reflect ASLC holding capacity under an all Group 2 pinniped scenario.

Our captive Steller sea lions are arguably the most important ambassadors for the Alaska SeaLife Center, the community of Seward, and the entire Alaska region. As such, great care is always taken to ensure their comfort and safety. All three animals have been trained to move into separate enclosures, both as a part of the proposed breeding study and as a part of their own health care. The male has received additional training in the form of a recall signal (a police whistle) which indicates that he should separate into an adjacent enclosure where he receives primary reinforcement while the door is secured by husbandry staff. He has responded without hesitation each time the recall signal has been given (approximately 7 times per week) since the original completion of the behavior, and continues to do so even when occupying the same enclosure as the females. The ASLC husbandry staff believes his behaviors leading up to the breeding season are a good indicator that he will continue to respond to the recall signal regardless of season.

**Comment [NMFS3]:** Would sedation be administered as described below if he won't separate during a copulation attempt if a female is potentially being injured?

Animals are monitored in person or via closed circuit television all day, every day, to ensure that females are not being injured by the male. Additional husbandry, veterinary, and research staff members are on call 24 hours a day to respond to any questions or concerns onsite staff may have. As an added precaution, all husbandry and veterinary staff have been trained to administer

**Comment [JJJ4R3]:** Husbandry staff will separate animals by utilizing a recall, operant conditioning, or distractions such as a high pressure water hose, air horn, etc. Sedation drugs are available only as a last resort administered at the discretion of the attending veterinarian.

sedation drugs via pole syringe, blow dart, and CO2 rifle, in the event that the male does not respond to a recall signal.

**Comment [NMFS5]:** What precautions are in place to prevent drowning?

Following breeding season, animals will be housed separately if deemed necessary by husbandry, veterinary, and research staff. This could be as simple as moving an animal into an adjacent pool within a habitat (e.g. from ODL 8 to 6), or it could involve moving animals to entirely different habitats within the ASLC (e.g. from SSL habitat to ODL). We have multiple wheeled cages suitable for transferring the largest of animals throughout the facility.

**Comment [JJJ6R5]:** All habitat doors will be open so that animals can be secured in dry holding. Sedation drugs would be administered at the discretion of the attending veterinarian as a last resort method for mitigating male aggression and separating animals. As soon as this separation is achieved, reversal agents can be administered.

As parturition nears, females may be secured in separate dry runs during times when husbandry staff is not present. Following parturition, the dry runs will be modified with doors that will restrict pups while allowing females to access water. A small wading pool will be placed in the dry holding area, at the discretion of husbandry or veterinary staff, when the pups reach 1-2 weeks of age. Pups will be allowed supervised access to this pool, and mothers will continue having free access to other pools without the accompaniment of their pup. Pups will be allowed to join their mothers in larger pools only after husbandry, veterinary, and research staff are satisfied that they have demonstrated sufficient mobility. Both pairs of mothers and pups will be granted supervised access to one another at the discretion of husbandry staff. Pairs will be separated if mothers become agitated by the presence of one another. Pups will be gradually weaned onto solid food utilizing protocols established by leading marine mammal breeding facilities (e.g. Sea World), when husbandry, veterinary, and research staff determine it is appropriate.

**Comment [NMFS7]:** Where will pups be held if a mother fails to nurse or otherwise rejects her pup and the pup has to be hand-raised?

c. Research induced harm to mother/fetus/pup-

The health and well-being of the animals in question is, and always has been, paramount. The proposed research requires captive sea lion physiology which mimics that of free-ranging animals as closely as is possible within a captive setting. Previous studies examining physical, metabolic, and hormonal changes during pregnancy and lactation among California sea lions have indicated that these types of research procedures (e.g. restraint, anesthesia, blood draws, ultrasound, etc) can be carried out without harming mother, fetus, or pup (Grieg et al, 2007; Williams et al, *in press*).

**Comment [JJJ8R7]:** Rejected pups will be housed in enclosures at ASLC. The ASLC staff have successfully reared orphaned wild Steller sea lions, Pacific harbor seals, spotted seals, Pacific walrus, and sea otters, as well as juvenile and adult members of several other pinniped species.

**Comment [NMFS9]:** Please provide a copy of this paper to our office.

**Comment [JJJ10R9]:** See attached.

d. Overwhelming or conflicting studies-

All research performed at the ASLC receives absolute oversight from an Institutional Animal Care and Use Committee (IACUC), and additional supervision from our husbandry, veterinary, and research departments. These entities help manage our captive programs to ensure that research activities will never trump the welfare of the animals. The ASLC IACUC reviews and approves all procedures involving our captive animals prior to initiation of the study. The proposed breeding study was approved by the IACUC on May 4, 2007.

To the greatest extent possible, animals are trained to submit voluntary samples (e.g. saliva, morphometric measurements, blubber ultrasounds, etc) so that procedures will not induce stress. When anesthesia is required (e.g. blood draws, blubber biopsies), multiple procedures are performed simultaneously to reduce potential stress. Previous research has shown that the type of anesthesia used does not induce a stress response, acute or otherwise (Mashburn and Atkinson, 2004). Please see attached table for an illustration of number of voluntary versus restraint takes.

The take table submitted with our amendment application indicates the maximum number of takes our animals could be exposed to each year, during the course of a 5 year study. That is not to say that each animal is exposed to each take, each year. Rather, each animal is exposed to a combination of the permitted takes during each year and exposed to each type of take over the course of the 5 year study.

Pregnancy would interfere with caloric restriction (i.e. fasting) and stress mimicking (e.g. ACTH, etc) studies, and therefore these procedures will not be performed during gestation. In addition, fasting studies will not be performed on lactating animals to ensure their pups are receiving adequate nutrition.

Current studies focus on gathering physical, metabolic, hormonal, and immunological data throughout the lives of our captives. ASLC scientists anticipate gestation and lactation will be reflected in the data obtained, and in fact this change is one of the things we wish to study. We have over a decade of information about these individuals in a variety of scenarios (e.g. immature, mature, fasting, gorging, stressed, relaxed, etc), but we have yet to examine what occurs to baseline data during gestation. Two recent ASLC funded studies have performed similar research (e.g. physical, metabolic, and hormonal change) and procedures (e.g. restraint, anesthesia, blood draws, ultrasound, etc) on pregnant California sea lion (*Zalophus californianus*) females without harm to mother, fetus, or pup (Grieg et al, 2007; Williams et al, *in press*). The proposed breeding study is a continuation of our previous work, utilizing sound science and techniques tested on surrogate species, which will provide valuable insight into breeding biology of an endangered species.

## 2. Relevance to recovery:

Very little is known about the physiology of the annual reproductive cycle for Steller sea lions. They appear to have low reproductive success relative to other pinnipeds, in that nearly 100% of adult females are believed to be impregnated each year, 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 or lactating individuals may be more susceptible to reproductive failure.

The ASLC captive research program, including the proposed captive breeding study, has been designed to yield long term baseline data as well as innovative non-invasive techniques which can be compared to, or utilized on, free-ranging populations. This work closely follows directives found in the Steller sea lion recovery plan (NMFS 2007). For example:

Task 1- “Baseline population monitoring is necessary to support all of the recovery actions. They describe the status and trends, vital rates, and health and body conditions of individuals... The SSL Recovery Plan also calls for improvement and/or development of methods with which to establish reproductive rates; provision of indices of health and status using chemical methods; and improvement of live capture methods and non-lethal sampling techniques.”

Task 3- “calls for researchers to use new technologies that reduce disturbance, potential mortality, and the need for invasive methods.”

Task 4- “The SSL Recovery Plan calls for analysis for agents or diseases with potential to affect the survival, growth, reproductive, etc. effects on SSLs. Research methods that provide these data include blood sampling, fecal samples, tissue sampling...”

Task 5- “Baseline population monitoring is necessary to support all of the recovery actions. They describe the status and trends, vital rates, and health and body conditions of individuals... The SSL Recovery Plan also calls for improvement and/or development of methods with which to establish reproductive rates; provision of indices of health and status using chemical methods; and improvement of... non-lethal sampling techniques.”

Success in the proposed breeding study will produce essential baseline information, and allow for the analysis of archived samples collected from free-ranging animals within the context of the animals’ actual physiological status at the time of collection. For example, recent ASLC captive studies have developed methods which have been highly successful in documenting physiological activity, such as response to predation, in free-ranging animals (Mashburn and Atkinson, 2007)

Additional information on how the proposed research is relevant to wild populations and their recovery can be found throughout the background section of our original application.

### 3. Transfer/import/export

**Comment [NMFS11]:** It would be helpful if it was explained how the proposed breeding study will specifically address or achieve these objectives (i.e., a reiteration of methods and study design that will logically explain how the results will have bearing on these recovery objectives). If you are developing methods, shouldn't this be done on a surrogate species first? Have all methodologies and assays been worked out for this study?

**Comment [JJJ12R11]:** As stated in our applications, very little information exists which describes the vital rates and health and body conditions of pregnant or lactating Steller sea lions. Previous captive breeding at foreign facilities has been done under “rookery pool” scenarios and have not produced published data. The work we have proposed to do (e.g. collecting urine, scat, saliva swabs, etc) is currently being done with these same captive animals, and has also been done with surrogate species (e.g. California sea lions. Williams et al, *in press*; Grieg et al 2007). Examining animals that are known to be healthy and pregnant (i.e. ASLC captives) will allow for establishment of normal baseline parameters which can be compared to free-ranging animals.

**Comment [JJJ13R11]:** Mashburn and Atkinson (2004,2007, 2007) Richmond, et al. (2005), Litz, et al. (2005, 2006) and Myers, et al (2007) have shown that chief physiological components of homeostatic, adrenal, and reproductive function have been successfully validated for use to describe the physiology of both captive and free-ranging Steller sea lions. The methodology developed in the aforementioned studies has also been used to successfully describe the longitudinal effects of predation on free-ranging animals (Mashburn and Atkinson, 2007). We feel that assay and testing systems currently in place grant the ability to study captive animals and can be easily modified for use in the field as well.

**Comment [JJJ14R11]:** Pages 4-7 of the original amendment application provide detailed explanation for how the proposed breeding study fulfills objectives set forth in the Steller sea lion recovery plan.

Our captive animals are 13 years old and well within normal breeding age range. We expect our females to be impregnated the first year by our male via natural means, however if this is not successful we will need to explore other options. Anecdotal evidence from facilities with successful breeding programs of related pinnipeds (e.g. Sea World, Long Marine Lab, etc) seems to indicate that they are more often successfully bred under “rookery pool” scenarios with multiple males and females in proximity to one another. We would seek to add additional animals to our collection only if initial captive breeding efforts fail to produce pregnant animals. Transferred animals could be on loan and therefore may need to be returned to originating facilities. No current ASLC animals, nor any progeny from this study, will be released into the wild.

The ASLC does not currently possess permits to transfer or import/export additional live Steller sea lions. However, ASLC will work closely with OPR to ensure that the proper permits and agreements are obtained prior to transfer or import/export of SSL from/to Mystic Aquarium, Oregon Zoo, and/or Vancouver Aquarium.

ASLC included a written MOU with Mystic Aquarium with our amendment application. Additional MOU’s with Vancouver Aquarium and Oregon Zoo will be submitted with any transfer or import/export permit applications tendered in the future.

**Comment [NMFS15]:** If you would like to include these scenarios in this amendment so that they could occur WITHOUT a MAJOR amendment, then we need to identify all potential animals now, documentation of their origin, and the maximum number by sex that would be held at ASLC.

**Comment [JJJ16R15]:** We cannot sufficiently address this issue in the current application. Please remove animal transfer/import/export from our application.

#### Marine Mammal Commission (MMC)

1. Based upon the Commission’s understanding from the Animal and Plant Health Inspection Service, the permit holder does not currently have sufficient space to conduct a Steller sea lion breeding program.

**PR1: Please explain how your facility would meet APHIS space requirements for the additional animals associated with the proposed captive breeding.**

See note 1 above, attached table, and schematic drawings.

2. Also, we note that, in addition to studying the physical, metabolic, hormonal, and immunological changes during gestation and lactation, the applicant will continue to conduct currently authorized research “deemed harmless to mother, fetus, and pup” on the subject females. It is unclear whether and, if so, the extent to which these multiple studies might bias the results of the proposed breeding study.

**Comment [NMFS17]:** What if all pups born are male? We have to be able to account for their long-term maintenance. What other facilities have the capacity to take 4 adult males?

**Comment [JJJ18R17]:** ASLC can permanently house 5 sexually mature males simultaneously, as indicated in the attached table.

Most current studies are descriptive in nature and will not influence the breeding study. Studies that could interfere with pregnancy or lactation are described in note 1 above and in the attached table.

3. Finally, the applicant should explain more fully the relevance of the proposed breeding study to the recovery of the wild population of Steller sea lions.



See note 2 above.

USDA Animal and Plant Health Inspection Service (APHIS)

4. The facility does not have room for additional Steller sea lions, as at least one of the ODL pools identified is not large enough.

**PR1: Please explain how your facility would meet APHIS space requirements for the additional animals associated with the proposed captive breeding.**

See note 1 above, attached table, and schematic drawings.

5. There does not appear to be a valid reason to breed this endangered species in captivity, unless they are being bred for future release. As this process has not proven successful with marine mammals, use of these animals, already subject to a large number of experimental protocols, will not benefit the species or the animals themselves.

**PR1: Please explain how the captive breeding would benefit Steller sea lion conservation.**

See note 2 above, and the Draft Steller sea lion recovery plan (NMFS 2006)

6. The issue of export and import of animals was not addressed. Any approval of this protocol is not permission for such movements. Those movements require other permits or approval.

**PR1: Please explain what other permits or approvals have been obtained or sought in relation to this proposed activity.**

MOU's and animal ID's will be submitted with a minor amendment request, should the desire to transfer animals arise. See note 3 above.

**Comment [NMFS19]:** Unless these transfers (animal IDs, origin, location, etc.) are described here, it will be a major amendment.

7. If the animals were impregnated, all other experimentation and sampling should be discontinued, as pregnancy will interfere (potentially) with other studies, and other manipulations would endanger the pregnancy.

**PR1: Please fully discuss issues related to effects of pregnancy on specific studies and vice versa.**

**Comment [JJJ20R19]:** See comment on page 5.

See note 1 above. Most current studies are descriptive in nature and will not influence the breeding study. Studies that could interfere with pregnancy or lactation are described in note 1 above and in the attached table.

8. With such a small sample size, it is very doubtful that any significant studies could be performed/data analyzed.

**PR1: Please explain how the sample size would provide statistically robust data or otherwise be applicable to the population at large.**

Statistical power is not a concern because we are not conducting a controlled or manipulative experiment- the proposed studies are observational. Very little is known about the immune,

hormonal and metabolic systems of SSL during pregnancy and lactation. Studying our captive population will provide baseline information on animals that are known to be healthy. This data can later be compared to free-ranging populations in order to detect differences that may be indicative of reproductive failure. Holmes et al (2007) have shown that decreased reproduction is at least partially responsible for the continued decline/slow recovery of the western stock SSL. We believe that understanding the physiological mechanics of pregnancy and lactation, which is most efficiently accomplished in a captive setting, will lead to insights in managing free-ranging populations. The exact nature of these insights is unclear, but the exact nature of the reproductive problems in the western stock is also unclear. For example, we don't know if females are aborting fetuses, abandoning newborn pups, failing to implant embryos, not becoming pregnant as frequently as they once did, or if they are experiencing a combination of these and other factors.

### NMFS Reviewers

9. Any animals that may be imported or received from cooperating institutions must be identified by animal ID, age/sex, and supporting documentation of the animal's origin (e.g., collection permits and methods; supporting letter from facility that animal was born in captivity, etc.) must be submitted with the permit application.

Animal ID's and supporting documentation will be submitted with a request for a **minor amendment** to the current permit, should the desire to transfer animals arise. See note 3 above.

**Comment [NMFS21]:** Please see comments above re: major amendment.

**Comment [JJJ22R21]:** See comment on page 5.

10. Any animals that may be exported must be identified as well, and the receiving facility must provide certification as required in the permit application (section C.5).

**All facilities are USDA/APHIS licensed and inspected. They are appropriate for the care and housing of marine mammals, including endangered Steller sea lions.**

**Comment [NMFS23]:** The question pertains to foreign facilities if animals were to be exported, which requires supporting documentation from the foreign government.

**Comment [JJJ24R23]:** See comment on page 5.

11. The permit applicant should provide letters of agreement from the cooperating institutions.

Supporting documentation will be submitted with a request for a **minor amendment** to the current permit, should the desire to transfer animals arise. See note 3 above.

**Comment [NMFS25]:** Please see comments above re: major amendment.

**Comment [JJJ26R25]:** See comment on page 5.

12. In addition to the information provided in the background section of the application, additional information with citations should be given regarding what captive breeding has been accomplished to date and what information has been gained from captive breeding of Steller sea lions and other sea lions, such as California sea lions in zoos and aquaria in the U.S. and abroad. More background information on what research on reproduction, gestation, lactation, and pup growth and development has been conducted and would be concurrently studied in the wild population of Steller sea lions should be added to this section. For example, page 4 of the application states: "Reproduction: Despite several studies describing the reproductive biology of Steller sea lions...." These studies and any published information (e.g., Atkinson 1997,

Reproductive biology of seals; Robeck et al. 2001, Reproduction, in CRC Handbook of Marine Mammal Medicine) should be described and referenced.

See note 2 above.

**13.** The objectives, hypothesis, and justification section is not complete. The applicant should refer to the application instructions and provide a revised version of this section and completely address the information required. For example, the applicant must justify why this research cannot and should not first be carried out with a surrogate species such as California sea lions. It is not clear that all methods have been well established for this study and that this study is justified or necessary for this species. In addition, page 7 of the application states that the animals at the ASLC are “highly trained for stress-free, voluntary participation in this very type of research” yet this is not further qualified as to what types of research this includes. For example, page 10 of the application indicates that transrectal/transvaginal ultrasound may require sedation/restraint, contrary to this statement.

Similar research and procedures on California sea lions (*Zalophus californianus*) have established that these types of research procedures (e.g. restraint, anesthesia, blood draws, ultrasound, etc) can be carried out without harming mother, fetus, or pup (Grieg et al, 2007; Williams et al, *in press*). Please refer to note 2 above, the previously submitted amendment application, and the Draft Steller sea lion recovery plan (NMFS 2006) for justification for this research. A table has been provided to clarify procedures which require restraint.

**14.** All activities and all changes to activities in take table must be clearly justified.

All current research takes are listed in black “normal” text within the take table, while new takes or clarifications for pregnant/lactating animals are listed in blue italics. Justification for these takes/clarifications can be found in the amendment application. Suggested alterations indicated with strikeouts were not adequately justified and are withdrawn.

**15.** It is not clear from the application that the ASLC has adequate space for these additional animals, or how the ASLC proposes to manage the movement of animals within the ASLC or among various facilities to facilitate space. The information in the application is vague. All possible scenarios for how this would be accomplished must be described in order for the application to be properly evaluated. The maximum number of animals by age/sex that may be held per pool must be described.

Please see note 1 above and the attached table.

**16.** All possible contingency plans for animal management (including emergencies) should be well thought out and provided prior to a decision being made on issuing a permit, including contingency plans and where animals will be located during breeding, gestation, birth, lactation, and weaning. For example, on page 18, the application states that animals will be separated if deleterious behavior is

**Comment [NMFS27]:** More descriptive background information on what has been done to date and what is known/published was requested.

**Comment [JJJ28R27]:** Unfortunately, much of the recent information gained about the reproductive biology of Steller sea lions was obtained in the 1970s and the 1980s from captured and/or sacrificed free-range animals (Pitcher, et al, 1998; Pitcher and Calkins, 1981). While this data is extraordinarily important to the understanding of the biology of the animal, captured and sacrificed specimens represent a snap-shot of the physiology in one discreet moment and can not be examined within the context of a process. Thus, physiological events and their timing are inferred and not well understood. While scientists are able to make estimates of processes, such as the timing of estrus, fertilization and implantation, we have not been able to investigate these events as interactions within the whole living animal (Iwasa and Atkinson, 1996; Hobson and Boyd, 1984). Very few studies have been undertaken in which living animals were used to provide longitudinal data. The sample sizes are small and the methodology invasive (i.e. blood sampling) (Raeside and Ronald, 1981; Gardiner, et al., 1999). In both the mentioned studies, only the period of late gestation was monitored and it must be noted that there were no disrupted pregnancies. The use of non-invasive techniques (e.g. collection of scat, urine, saliva, etc) will allow for a much more comprehensive analysis of the processes that occur during successful reproduction. This will allow for greater depth of understanding and can allow us to begin to identify those animals in which the temporal events are afunctional.

observed (under parturition mitigation) but is not clear how, when, or where the separation will occur.

Please see note 1 above.

- 17.** Would recipients (e.g., Mystic Aquarium) of offspring or other ASLC animals obtain their own permit to receive these animals or would the animals continue to be held under ASLC's permit at another location?

We do not have a preference for which permit transferred animals are held under.

- 18.** On page 18 of the application, please indicate clearly what procedures require restraint, sedation, anesthesia, or can be performed voluntarily.

Please see attached table.

- 19.** On page 18, in description of breeding mitigation, has training for separation been accomplished? What is the likelihood that this could actually be accomplished during mating, when the adult male will likely not be responsive for obvious reasons? Have you consulted with Mystic Aquarium on the incident with their adult male attacking and killing a female during a mating attempt?

ASLC staff has consulted with Mystic Aquarium about the death of one of their adult females during the breeding season. A thorough necropsy was performed in an attempt to determine exact cause of death. The evidence did not support the claim that "their adult male attack[ed] and kill[ed] a female during a mating attempt". Lacerations and puncture wounds appeared to be old and necrotic. Food was offered but not consumed in the 12 days prior to death. Drowning was inconclusive. The final diagnosis was Bacteremia, drowning secondary to exhaustion.

As stated previously in this application, the animals at ASLC are conditioned to move into separate enclosures and they participate in daily husbandry exams. Extended bouts of anorexia have not been common with our animals. Any animal, male or female, that appears to be compromised would be housed separately until Veterinary and Husbandry staff determines that their condition would allow them to be housed in the same enclosure as the other animals.

- 20.** On page 20, the application states that the pups will have access to their mothers unless a short term project dictates. This should be clarified and a list of what studies this would include should be provided.

Un-weaned pups will have daily access to their mothers. See attached table for additional clarification on short term separation.

- 21.** At what age would pups first be given gas anesthesia?

Gas anesthesia will be administered at the discretion of the attending veterinarian and mammal curator, following in-depth discussion with the Principle Investigator. Free-ranging Steller sea lion pups are commonly anesthetized at >5 days of age at rookeries throughout Alaska, and in Oregon and California (John Maniscalco, personal communication). As with all our captive

pinnipeds, pups will be conditioned to submit voluntarily samples for as many takes as possible. Requested takes requiring anesthesia include blood draws and radiographic examinations.

- 22.** There is a possibility that an adult female could be killed by the male but this accidental mortality has not been requested.

We recognize the risks associated with breeding and rearing captive animals, and requested “one (1) lethal take of a live-born Steller sea lion during the proposed study.” This is not an age specific request. Rather, it is meant to cover all animals participating in the proposed study.

- 23.** What is the status of the IACUC review and approval for the captive breeding and research on females and pups? The application says that the current IACUC letter is on file but the study has not been permitted yet.

A new Animal Use Protocol (AUP) for captive research, including the proposed breeding study, was approved by the ASLC IACUC on May 4, 2007. Please see the attached approval letter.

#### The Humane Society of the United States (HSUS)

- 24.** It is not clear that this applicant has sufficient space at their facility to properly house animals under APHIS guidelines. As noted, the NMFS cannot issue a permit until and unless the facility received all necessary approvals from APHIS. This permit application is premature. 50 C.F.R. § 222.308(c)(10) (to issue a permit, NMFS must consider “how the applicant’s needs, program, and facilities compare and relate to proposed and ongoing projects”).

See note 1 above, attached table, and schematic diagrams.

- 25.** There are no specific hypotheses being tested, making it difficult for NMFS or the public to determine how this proposal will contribute to a research need identified in a recovery plan, contribute “significantly” to understanding of the species’ biology or conservation issues, or fulfill a “critically important research need.” Id. § 216.41(b)(5)(iii).

We are testing whether “physiological measures of endocrine, immune, and metabolic systems, as well as morphometrics, can be quantified to predict health of an individual.” The application submitted by ASLC is for an amendment to an existing permit, and therefore only lists the new hypothesis being tested by the proposed breeding study. Please refer to our previous application materials, approved in permit 881-1745, for further clarification on additional hypotheses.

- 26.** Justification for various procedures and clear discussion of the potential consequences of its activities are lacking. For example, Page 11 asserts that they “have never heard any reports of anesthetic symptoms or other complications in pups of immobilized Steller sea lions” and then cite a single anecdotal observation at Lowrie Island. They should conduct a thorough literature search of anesthetic effects in this and similar species rather than relying on this single bit of anecdotal evidence. The application should be supplemented.

Isoflurane is a halogenated volatile anesthetic that has been safely used for decades on a variety of animals, including those that are pregnant (Williams et al, *in press*). 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 (e.g. milk), 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).

**27.** We also find it interesting that this application casts doubt on the applicant institution's proposal to study maternal condition, lactation and reproduction in wild animals (see above File #881-1890). This application states that studies of captive animals are better in many respects because "associated handling stress [with free-ranging animals] could perhaps disrupt the reproductive events being studied." (page 4) Applicant ASLC cannot have it both ways. Either the wild studies provide valuable insight into reproductive members of the population with little stress and risk to reproductive and/or nursing mothers as stated in the earlier application or they do not.

Researchers cannot learn everything about Steller sea lions by only studying captive or free-ranging populations- they must study both. Research programs such as ours are designed to include both free-ranging and captive components that are complimentary in nature and serve to validate results obtained. As we stated in our application, there are some advantages to captive studies. For example, captive animals are fully habituated to human presence and have been conditioned to give voluntary, stress free samples (e.g. morphometric and ultrasound measurements, saliva and scat samples, etc.), while the same procedures performed on free-ranging counterparts would require restraint and anesthesia. Studying our captive population will provide baseline information on animals that are known to be healthy. These data can later be compared to free-ranging populations in order to detect differences that may be indicative of reproductive failure. Holmes et al (*in press*) have shown that decreased reproduction is at least partially responsible for the continued decline/slow recovery of the western stock of SSL. We believe that understanding the physiological mechanics of pregnancy and lactation, which is most efficiently accomplished in a captive setting, will lead to insights in managing free-ranging populations.

**28.** The summary charts accompanying the application show a number of alterations under takes/animal/year, evidenced either by newly bolded language or strikeouts of previous, smaller numbers. The justification for the numbers is not provided in the application and there is certainly no justification provided for changing the original verbiage (e.g. the strike-outs indicate a change of thrice weekly swabs to daily swabs, drawing blood changed to four times a year instead of two). The justification should be, but is not, adequately explained in the application.

All current research takes are listed in black "normal" text within the take table, while new takes or clarifications for pregnant/lactating animals are listed in blue italics. Justification for these takes/clarifications can be found in the amendment application. Suggested alterations indicated with strikeouts were not adequately justified and are withdrawn.

**29.** Further there is insufficient justification provided for breeding additional long-term captives as a means of providing insight into free-ranging animals. Their restricted mobility, artificially altered diets and additional artificialities that are a necessary consequence of captivity are likely to limit the insights that can be gained.

Scientists can alter nearly every aspect of captivity, thereby eliminating many of the variables that influence free-ranging populations. By eliminating many of the variables of day to day existence, we can assess the affect each variable has on an individual. For example, we can control the amount of high fat fish that are consumed, and then observe differences in the blubber layer of the animal. In the same respect, we will be able to control many of the variables of pregnancy and lactation and get baseline data to compare to free-ranging populations.

**30.** This application provides insufficient justification of the need for captive breeding of this species, particularly if animals cannot be properly maintained in the facility that has continued to justify their captivity. 50 C.F.R. § 222.308(c)(10) (to issue a permit, NMFS must consider “how the applicant’s needs, program, and facilities compare and relate to proposed and ongoing projects”).

The Steller sea lion recovery plan directs scientists to utilize captive animals to develop positive and progressive studies to determine how nutritional and reproductive requirements affect fecundity. It currently is not possible to study these requirements, or physiology and immunology during gestation and lactation, without impregnating animals. Please see note 1 above, attached table, and schematic drawings.

**31.** The applicants state that, if permitted, this activity “may require” (page 3) the transfer of up to 4 adult animals “i.e. 1 male and 3 females” (page 7 and 17) to other captive display facilities. The reason is not explained. Is it for space reasons? Concerns over aggression? A preference for keeping younger animals? No valid answer, nor indeed any answer, is provided in the application that would justify producing four newly born permanent captives, thus necessitating the transfer of four current captives.

We desire that all captive SSL maintained in the US be utilized in research directed at the recovery of the free-ranging population. As such, we seek the flexibility to work with other institutions to ensure that each facility and research project has the appropriate age/sex animals.

**32.** The applicants state that captive born offspring of long-term captive mothers “may participate in valuable scientific studies.” The basis for and nature of the studies are entirely unclear. Page 12 simply states that “pups produced during this study will play an important and *evolving role* in fulfilling the ASLC research mission.” There should be a clear and pressing need for a specific sort of research to justify producing more captive animals that will require their transfer or the transfer of other animals to outside facilities in the process. Again, no specific hypotheses are provided for testing.

**Comment [NMFS29]:** Please expand.

**Comment [JJJ30R29]:** Our response is a reflection of our desire to manage captive Steller sea lions at all facilities as a “population”, which can be transferred among facilities as research and programmatic needs dictate, instead of static individuals tied to a specific facility. We will address this topic in a future major amendment request.

Research objectives evolve parallel with current knowledge of the greater scientific community. For example, many additional research tasks were added between the issuance of the 1992 and 2006 Steller sea lion recovery plans. The proposed study seeks to study adult females during gestation and lactation, and to begin collecting basic condition assessment data from any pups produced.

**Comment [NMFS31]:** A review of this “current knowledge” should be provided in the background information.

**33.** Further the number and purpose of animals involved in inter-institutional transfers is confusing. Though it is clearly stated on pages 7 and 17 that four animals may be transferred, the verbiage on page 21 indicates that the transfers involve four adults, the production of 4 pups “as well as 3 adult females transferred from Mystic and/or Oregon Coast Aquarium, or imported from Vancouver Aquarium.” Which animals are being proposed for transfer to substitute for or add to which current captives? The application is not entirely clear.

**Comment [JJJ32R31]:** Before abnormalities in reproductive events can be recognized as such, the parameters of normal reproduction need to be defined. It is therefore of paramount importance that basic reproductive patterns in normal and healthy animals be investigated. 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 only an estimated 63% of those females deliver pups (Pitcher and Calkins, 1981). These reproductive failures include fetal resorption and spontaneous abortion, pseudopregnancy or failure to conceive, and interference during embryonic diapause or implantation. The mechanisms that drive these failures are poorly understood because the mechanisms that drive successful pregnancy are, in themselves poorly understood in these animals. There are several specialized features of reproduction that appear to be common (although not limited) to most species of pinnipeds: seasonality, high degree of synchrony, embryonic diapause/delayed implantation, and post-partum estrus. Mechanisms of control and interrelationships of these components of pinniped reproduction are not very well understood in any species (Paria et al., 2001; Basset et al., 2001; Badiwaik and Rasweiler, 2001; Renfree and Shaw, 2000; Martin, et al., 1999) which should only serve to emphasize the importance of a detailed description of reproductive events in a threatened species, such as the Steller sea lion. The complete estrus cycle, both successful (resulting in pregnancy, gestation and parturition) and non-successful, and the interdependence of reproductive hormones upon one another still needs to be determined. Steller sea lion reproduction is highly seasonal and synchronous. This suggests a reproductive system that is under very tight hormonal control probably cued to photoperiodic and metabolic signals (Pitcher et al., 1997) as has been suspected in other species of pinnipeds (Atkinson, 1997; Gardiner et al., 1999). They also have a low birth rate, indicating considerable maternal investment in the production of offspring. Given these characteristics of reproduction, disturbances in the external environment coupled with population ... [1]

See note 3 above.

**34.** Page 21 makes it appear that 3 current ASLC animals will be transferred (presumably after breeding, but this is not clear) and the facility wishes to import an animal from Mystic Aquarium. What is the reason for the transfer of additional animals from other institutions and how does this relate to the studies proposed in the permit? For example, are some current captives being transferred so that others can be bred? If so, then this conflicts with the activities described in the permit.

We expect our females to be impregnated by our male via natural means, however if this is not successful we will need to explore other options to increase likelihood of impregnation. Anecdotal evidence from facilities with successful breeding programs of related pinnipeds (e.g. Sea World, etc) seems to indicate that they are more often successfully bred under “rookery pool” scenarios with multiple males and females in proximity to one another. We would seek to add additional animals to our collection only if initial captive breeding efforts fail to produce pregnant animals. Transferred animals could be on loan and therefore may need to be returned to originating facilities.

**35.** Are pups involved in the transfers and, if so, how does this affect the “studies” in which the ASLC proposes they will participate to further the mission of the organization? Are pups being bred to increase the number of Steller sea lions in captive display facilities, with the research being somewhat secondary in nature?

No, research is our primary goal.

#### Public commenter

**36.** As stated in the application, the pregnant female Steller sea lions will undergo research procedures which could involve stress, forcible restraint, and anesthesia. These procedures could cause the female to spontaneously abort a pup or have a stillborn pup. Should such a situation be counted against mortality listed for the permit? Will allowances be made to reduce research induced stress on the animals once they are determined to be pregnant?

**Comment [NMFS33]:** Again, if this is not explained in this amendment, any future transfers will be considered a major amendment.

**Comment [JJJ34R33]:** See comment on page 5.



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.

All research performed at the ASLC receives considerable oversight from an Institutional Animal Care and Use Committee (IACUC) and our husbandry, veterinary, and research departments. These entities help manage our captive programs to ensure that animal health is the top priority and that research activities will never trump the welfare of the animals. To the greatest extent possible, animals are trained to submit voluntary samples (e.g. saliva, morphometric measurements, blubber ultrasounds, etc) so that procedures will not induce stress. When anesthesia is required (e.g. blood draws, blubber biopsies), multiple procedures are performed simultaneously to reduce the number of times we handle the animals. Caloric restriction studies (i.e. fasting) will not be performed on gestating or lactating females. Stress mimicking studies (i.e. ACTH and TSH) will not be performed during gestation.

- 37.** Other Steller sea lion pups have participated in long term physiological studies (including the animals at the Alaska SeaLife Center who have participated for the past fourteen years as well as animals from the Vancouver Aquarium in British Columbia). How will the data gained from the potential pups be significantly different from data already gained?

The proposed study examines changes in physiological measures of endocrine, immune, and metabolic systems of adult female Steller sea lions during gestation and lactation. The pups produced during this study will provide an opportunity to further previous studies performed on existing captives by including techniques that were not available 14 years ago when they were originally collected.

- 38.** The pups will be the product of animals from the Steller sea lion [eastern] stock which is currently reported to be experiencing a population increase. How will data from the [eastern] stock assist in determining the reason for the [western] Steller sea lion stock decline?

While the eastern stock may be increasing, the rate of increase is not as high as in other pinnipeds within the same area (e.g. *Zalophus californianus*). It should not be assumed that an increasing population is completely healthy or growing at an optimum rate. We are examining health indices thought to be universal between the populations. We cannot assess difference between the stocks until we determine what is “normal” among healthy animals.

- 39.** The female animals are fourteen years old which is a late age to be having a first pup. How will physiological data from their pregnancies be applied to wild animals that usually have pups much earlier in life?

While we do know that SSL can begin reproducing much earlier than 13 years, we cannot say with certainty that there are not free-ranging animals reproducing for the first time at that age. The proposed study is not designed to look at differences in pregnant animals of different ages. Instead, we will examine the physical, metabolic, immune, and hormone differences in pregnant

vs. non-pregnant and lactating vs. non-lactating animals. We believe that understanding the physiological mechanics of pregnancy and lactation, which is most efficiently accomplished in a captive setting, will lead to insights in managing free-ranging populations.

- 40.** What measures are in place to adequately protect the female Steller sea lions from a potentially violent and/or deadly breeding interaction? Does the potential data gained from breeding outweigh the risk of breeding the animals in a captive setting?

See note 1 above.

- 41.** This project could potentially result in four new animals living in captivity. Are there animals already in captivity at other facilities that could be used for this research? What about the potential use of Steller sea lions that are brought into rehabilitation centers throughout the Pacific Northwest and deemed inappropriate for release?

The ASLC is one of the only facilities in the world that maintains adult Steller sea lions, and has established what is arguably the most rigorous research program to study them. Our animals are reproductively intact, sexually mature, and in their prime. They are habituated to daily human physical contact and have been conditioned to submit to basic measures of health and condition since they were approximately one month old. Scientists have monitored their physical and hormonal development into adulthood, and propose to continue monitoring these parameters throughout gestation and lactation. Data collected will be compared to the long term dataset on these animals as well as their free-ranging counterparts. The comparisons we are attempting to make (changes during pregnancy and lactation vs. long-term data previously collected) cannot be made using animals from other institutions. ASLC scientists have established what “normal” physical, metabolic, and hormonal parameters are for our captives. Other institutions are studying different parameters and have not been collecting the same type of data as ASLC.

Wild animals from rehabilitation facilities are not appropriate for the proposed breeding study. Steller sea lions of breeding age simply are not found in rehabilitation facilities.

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**Table 1. Alaska SeaLife Center pinniped holding facilities:** Pool surface area (SA), pool depth, and land surface area (DRA) were used to calculate maximum number of animals housed within an area under APHIS regulations for Group 2 pinnipeds.

Maximum number of animals is listed for current configuration only and does not reflect additional distinct dry resting areas that can be created within several pools by adding visual barriers such as fences, rocks, or foliage.

Pool	SA (ft <sup>2</sup> )	DRA (ft <sup>2</sup> )	Depth	Male SSL	Female SSL
SSL Habitat East	883	2000	16.7	1 and	11
SSL Holding	244	261	7	1	3
HS Habitat	371	650	17.5		5
154 A	133	146	6		2
154 B	133	146	6		2
ODL 5	314	572	6	1 and	2
ODL 6	314	572	9.5	1 and	2
ODL 7	134	163	6		2
ODL 8	1396	1425	9	1 and	19

**Table 2. Alaska SeaLife Center's Captive Pinnipeds:** Name, ID, age, and primary enclosure.

Animal	NOAA ID#	Species	Sex	Age	Primary Enclosure
Woody	NOA0005799	<i>Eumetopias jubatus</i>	M	14	SSL Hab
Sugar	NOA0005801	<i>Eumetopias jubatus</i>	F	14	SSL Hab / ODL 8
Kiska	NOA0005800	<i>Eumetopias jubatus</i>	F	14	SSL Hab / ODL 8
Pup 1	TBD	<i>Eumetopias jubatus</i>	M/F	Born '08	SSL Holding
Pup 2	TBD	<i>Eumetopias jubatus</i>	M/F	Born '08	ODL 6
Pup 3	TBD	<i>Eumetopias jubatus</i>	M/F	Born '09	ODL 5
Pup 4*	TBD	<i>Eumetopias jubatus</i>	M/F	Born '09	ODL 8*
Snapper	NOA0001074	<i>Phoca vitulina</i>	M	22	HS Hab
Chloe	NOA000	<i>Phoca vitulina</i>	F	12	HS Hab
Atuun	NOA0006236	<i>Phoca vitulina</i>	F	3	HS Hab
Qilak	NOA0006235	<i>Phoca vitulina</i>	F	3	HS Hab
Susitna	NOA0006233	<i>Phoca vitulina</i>	F	3	HS Hab
Siku	NOA0006281	<i>Phoca vitulina</i>	F	2	HS Hab
Tikanni	NOA0006280	<i>Phoca vitulina</i>	F	2	HS Hab
Shyla	NOA0006279	<i>Phoca vitulina</i>	F	2	HS Hab
<p>* If the first two pups are males we will modify the ODL 8 enclosure prior to our next breeding attempt such that the dry resting or social activity area shall be divided into two or more separate areas with sufficient visual barriers (such as fences, rocks, or foliage) to provide relief from aggressive animals.</p>					

**Table 3. Nature of takes requested in ASLC application to amend permit 881-1745.** Voluntary takes can also be performed while animals are under anesthesia for other procedures. For purposes of this table, separation is defined as research takes performed while holding mother and pup in separate enclosures (e.g. mother in squeeze cage while pup is in dry holding.)

Activity/Take	Voluntary	Restraint and Anesthesia	Mother/pup separation <4hrs
1. Measure body mass and morphometrics	X		
2. Blubber depth measurements using ultrasound	X		
3. Routine Blood Samples		X	X
4. Urine	X		
4. Feces	X		
4. Whiskers		X	X
4. Milk	X		
5. Bio-electrical Impedance Analysis (BIA)		X	X
6. Total Blood Volume- Evans blue dye		X	X
7. D2O Administration:		X	X
8. Nutritional Physiology: Food trials, dietary manipulation (includes live fish and dietary markers)	Fasting portion of study not performed on gestating or lactating animals.		
9. Epidermal and mucosal swabs and collections of saliva and other secretions; examine and measure external genitalia	X		
10. Blubber biopsies		X	X
11. Imaging: Video, photographic, radiographic, spectrophotometric, digital, and thermal imaging of animals	X		
12. Radiographic examination		X	X
13. Hormone Stimulation: ACTH (2 IU/kg) <u>or</u> TSH (0.1 IU/kg) administration	Not performed on gestating animals.		
14. Attachment and removal of instrumentation	X		X
15. Underwater foraging and drag trials	X		X
16. Bioenergetics: determine resting and active metabolic rate	X		X
17. DLW Validation:		X	X
18. Bioenergetics and Metabolic Development: Dietary marker administration + dry holding for up to 72 hours for post dosage fecal and urine sample collection	Unweaned pups to have daily access to mother		
19. Protein Turnover: Stable isotope and tissue metabolism: ingestion or IV administration of stable isotope 13C and 14N and post dosage blood sampling	Unweaned pups to have daily access to mother		
20. Stomach temperature telemetry		X	X
22. Transrectal ultrasonography		X	X
24. Copulation & Parturition	X		





Before abnormalities in reproductive events can be recognized as such, the parameters of normal reproduction need to be defined. It is therefore of paramount importance that basic reproductive patterns in normal and healthy animals be investigated. 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 only an estimated 63% of those females deliver pups (Pitcher and Calkins, 1981). These reproductive failures include fetal resorption and spontaneous abortion, pseudopregnancy or failure to conceive, and interference during embryonic diapause or implantation. The mechanisms that drive these failures are poorly understood because the mechanisms that drive successful pregnancy are, in themselves poorly understood in these animals. There are several specialized features of reproduction that appear to be common (although not limited) to most species of pinnipeds: seasonality, high degree of synchrony, embryonic diapause/delayed implantation, and post-partum estrus. Mechanisms of control and interrelationships of these components of pinniped reproduction are not very well understood in any species (Paria et al., 2001; Basset et al., 2001; Badiwaik and Rasweiler, 2001; Renfree and Shaw, 2000; Martin, et al., 1999) which should only serve to emphasize the importance of a detailed description of reproductive events in a threatened species, such as the Steller sea lion. The complete estrus cycle, both successful (resulting in pregnancy, gestation and parturition) and non-successful, and the interdependence of reproductive hormones upon one another still needs to be determined.

Steller sea lion reproduction is highly seasonal and synchronous. This suggests a reproductive system that is under very tight hormonal control probably cued to photoperiodic and metabolic signals (Pitcher et al., 1997) as has been suspected in other species of pinnipeds (Atkinson, 1997; Gardiner et al., 1999). They also have a low birth rate, indicating considerable maternal investment in the production of offspring. Given these characteristics of reproduction, disturbances in the external environment coupled with population reduction (such as was seen in the Western Steller sea lion population) can lead to a decline from which a species has difficulty in overcoming (Pichler and Baker, 2000). The essential task is to understand the mechanisms that govern the reproductive biology of healthy individuals and how these mechanisms are linked to normal changes in the environment. The most significant factor affecting reproductive studies lies in the difficulty in obtaining samples from known individuals with enough frequency to reveal endocrine patterns of reproduction.

Unfortunately, much of the recent information gained about the reproductive biology of Steller sea lions was obtained in the 1970s and the 1980s from collected free-range animals (Pitcher, et al., 1998; Pitcher and Calkins, 1981). While this data is extraordinarily important to the understanding of the biology of the animal, collected specimens represent a snap-shot of the physiology in one discreet moment and can not be examined within the context of a process. Thus, physiological events and their timing are inferred and not well understood. While scientists are able to make estimates of processes, such as the timing of estrus, fertilization and implantation, we have not been able to investigate these events as interactions within the whole living animal (Iwasa and Atkinson, 1996; Hobson and Boyd, 1984). Very few studies have been undertaken in which living animals were used to provide longitudinal data. The sample sizes are small and the methodology invasive (i.e. blood sampling) (Raeside and Ronald, 1981; Gardiner, et al., 1999). In both the mentioned studies, only the period of late gestation was monitored and it must be noted that there were no disrupted pregnancies. The use of non-invasive techniques (e.g. collection of scat, urine, saliva, etc) will allow for a much more comprehensive analysis of the processes that occur during successful reproduction. This will allow for greater depth of understanding and can allow us to begin to identify those animals in which the temporal events are afunctional.

Mashburn and Atkinson (2004,2007, 2007) Richmond, et al. (2005), Litz, et al. (2005, 2006) and Myers, et al (2007) have shown that chief physiological components of homeostatic, adrenal, and reproductive function have been successfully validated for use to describe the physiology of both captive and free-ranging Steller sea lions. The methodology developed in the aforementioned studies has been used to successfully describe the longitudinal effects of predation on free-ranging animals (Mashburn and Atkinson, 2007), thus we feel that we have many assay and testing systems currently in place that not only grant the ability to study captive animals but are easily modified for use in the field as well.