Responses to comments received on File No. 782-1889 application for National Marine Mammal Laboratory Steller sea lion research

May 17, 2007

Marine Mammal Commission

1. The applicant is proposing to use anesthesia in branding pups between the ages of five days and two months. It is not clear whether anesthesia also will be used in branding older pups, juveniles, and adults. As described in our general comments, we recommend anesthesia be used in all cases (i.e., involving pups, juveniles, and adults) with only limited, pre-justified exceptions;

Response: We only apply brands to anesthetized Steller sea lions, regardless of their age, and this procedure is explicitly stated in two separate parts of our permit application. In the research narrative on page 23 we describe that pups (<2 months old) are first anesthetized with isoflurane gas prior to brand application, and state on page 24 that: "Sea lions >2 months old (if not previously branded as pups) are branded under anesthesia following the same protocols." This procedure is also reiterated in our description of measures to minimize effects on page 35: "Hot-brands are applied only when an animal is under general anesthesia." As noted in Appendix 1 of our permit application, pups are selected for branding based on mass, not age *per se*. Given the timing of branding most pups selected will be at least one month old. However, since an umbilicus detaches at about 7 days, and it is conceivable that pups of that age could meet the minimum mass criteria we express the potential range of ages that might be handled to be consistent with the anticipated capture age range.

2. Darting adult female sea lions using Telazol, as proposed, involves a risk of mortality both from the drug and from drowning if animals enter the water before the drug takes full effect. We recommend that every precaution be taken when using this drug and that only veterinarians and biologists with significant experience in darting marine mammals be authorized to conduct activities involving its use;

Response: We agree, and it is already a standard practice reflected as a condition of Steller sea lion research permits that only highly experienced and well-trained personnel perform invasive procedures.

3. This and other applicants proposing to chemically sedate adult female Steller sea lions by darting, should identify the pup of an adult female sea lion that is targeted for darting, and after she is darted, observe the pup closely or place it in a portable pen until the procedure(s) on the mother are completed;

Response: When handling adult females with dependent pups, the welfare of the pups is of utmost concern. We presume that the comment is addressing young pups still on the rookery. Whether to observe the pup or hold the pup in a pen is a decision that is made on sight based on the best judgment of the attending veterinarian, the principal investigator and the conditions. With pups ≥5 months old, attempting to capture and hold the pup temporarily would likely be inappropriate.

4. The application does not, but should, discuss the potential effects of Telazol on the nursing pups of females injected with the drug. If adverse effects may reasonably be expected, appropriate research should be conducted to resolve uncertainties concerning possible effects and to develop methods to mitigate those effects, as appropriate;

Response: The potential for any sedative or drug to transfer to suckling offspring and thereby produce unintended indirect effects is a concern. The comment is correct that our application did not specifically address this potentiality. However, based on information provided by marine mammal veterinarians Dr. Pam Tuomi of the Alaska SeaLife Center, and Dr. Kimberlee Beckman (State of Alaska veterinarian), it appears that though the excretion of Telazol® in milk has not been specifically studied, there is evidence to suggest the likelihood of such effects on suckling sea lion pups is low. The two drugs that comprise the solution, tiletamine and zolazepam belong to the cylohexamine and benzodiazapine drug classes, respectively, the latter of which has been studied. When human infants were nursed soon after the mothers received a high-dose administration of diazepam (a benzodiazepine), plasma concentrations of diazepam in the infant were less than 10% of that of their mother, well below the concentration that could produce a clinical effect (i.e. sedation) or complication (Hale 1999; Lee and Rubin 1993). Ketamine, a drug most similar to tiletamine, is used as the disassociative anesthetic of choice for human infants and has a wide margin of safety with a very short half life, and short duration of action (20 to 55 minutes) in wildlife species studied. No specific studies on excretion in milk of ketamine or tiletamine were found in the veterinary literature, but in human neonates high lipid soluble drugs of low molecular weight that are non-ionized are estimated to administer 1-2% of the maternal dose to the neonate (Britt and Pasero 1999). Additional information provided in the ASLC application stated that Semple et al. (2000) reported tissue residues of Telazol in polar bears declined to trace levels within 24 hours post-immobilization, and even at 12 hours the highest concentrations were still well below a level that could produce an anesthetic effect. It thus seems unlikely that Steller sea lions could excrete enough Telazol in their milk to have an adverse effect on their pups.

According to information provided in the ASLC permit application there have been no studies on the teratogenicity of Telazol. However, although adult female Steller sea lions handled during the months of June–August might be pregnant, the embryo will be at the arrested blastocyst stage so any effects of Telazol will likely be negligible.

Britt R, Pasero C. 1999. Pain control: using analgesics during breast-feeding. Am J Nursing 99(9):20.
Hale TW. 1999. Anesthetic medications and breastfeeding mothers. J Hum Lact 15:185-194.
Lee JJ and Rubin AP. 1993. Breast feeding and anesthesia. Anesthesia 48:616-625.
Semple HA, DK Goerecki, SD Farley, and MA Ramsey. 2000. Pharmacokinetics and tissue residues of Telazol in free-ranging polar bears. J Wildl Dis 36(4):653-662.

5. The application states that an experienced marine mammal veterinarian will be present to carry out or supervise all activities involving the use of inhaled anesthesia. We believe this is already a standard practice. A curriculum vitae for the veterinarian(s) who would be involved in the research should be provided if one is not already on file with the Permits Office;

Response: Correct. Curriculum vitae of attending veterinarians are routinely provided.

6. This and other applications (Alaska Department of Fish and Game, Alaska Sea Life Center, Marcus Horning) state that Steller sea lions captured on floating platforms could be held for up to 12 hours while waiting to be sampled. The applicants should clarify what portion of time animals will be allowed to come and go from the platform, the maximum duration that they will be held on the closed platform, the maximum time that they will be maintained in holding cages before research procedures are initiated, what would be done to prevent their overheating, and why alternative capture or research protocols that require shorter holding times are impractical;

Response: Animals are free to come and go from platforms at all times except during the capture and holding process. The statement in the application that animals could be held up to 12 hours was a typographical error. Based on floating pen captures at Shilshole Marina in Washington, sea lions are rarely held for longer than 2 hours. The ultimate holding time will depend on a combination of number captured and procedures performed. Capture pens target large juveniles and adults and are open to the air. In the unlikely event of potential overheating animals can be sprayed with cold ocean water. Figure 1 shows sea lions associated with the capture structure in Kodiak harbor, Alaska. There are currently few capture techniques that can handle animals of that size, but techniques include pen captures, dart-delivered sedatives and at-sea net captures. The best choice of capture technique varies with study objectives and the timing and location of captures. Thus, our permit application sought authorization for all three capture techniques.

7. Insufficient information is provided concerning the proposed short- and long-term monitoring of animals, particularly mother/pup pairs, after release. This information is needed to allow reviewers to evaluate whether monitoring will be sufficient to assess the effects of branding/handling on the subject animals and should be provided prior to permit issuance.

Response: In the case of adult sea lions, virtually all requested takes involve the objective of attaching satellite-linked transmitters to the animals for the purpose of post-capture monitoring. It is not clear how the reviewer would assess the effect of branding/handling on a free-ranging animal without increasing the amount of disturbance or interfering with the animal. However, in the case of branding, pups are routinely observed following the branding procedure for as long as they are visible by the researchers, and at three sites with field camps in Alaska throughout the summer. The purpose of branding pups is based on the objective of resighting those individuals as much as possible throughout their lifetime. This provides updates on their survival and life history rates. Recent studies examining the survival of branded pups in Southeast Alaska report survival rates that suggest little or no affect on survival (Hastings et al. in review, cited in our application). In the case of handling of pups in remote sights the ability to position people for post-handling monitoring for extended periods of time does not exist. This would in many cases require the establishment of remote field camps so close to the sea lions that the long term disturbance is not justified. Prohibiting the handling of pups in these areas because of an unknown and unproven concern that the handling of the pups might have a positive or negative effect on their survival would be arbitrary and would not address the recovery actions in the Draft Steller Sea Lion Recovery

Plan.

Hastings, KK, TS Gelatt and JC King. *In Review*. Survival of Steller sea lion pups to 3-months post-branding at Lowrie Island, Southeast Alaska. J Appl Ecology.

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8. While there may be reasons to use hot branding for permanent identification, the activities in the multiple Stellar [sic] sea lion and northern fur seal applications do not warrant its use. The study design does not require and does not benefit from capture/release/recapture. All biochemical and body morphology do not require multi-year sampling of identified individuals. Any identification needed in one year to prevent resampling of the same animals can be served well by bleach or paint.

Branding should not be used unless it is the only means of identification possible. Since the protocols do not call for monitoring specific individuals as a key component, hot branding is not acceptable for these permits.

PR1: Please explain why your study cannot be accomplished without hot brands.

Response: The comment is incorrect that our Steller sea lion studies do not warrant hot-branding as a means of permanent identification, and we included a thorough justification of this method on pages 54-76 of our application as "Appendix 1. Justification for and Summary of Hot-iron Branding on Steller Sea Lions Including Branding on Other Pinnipeds." This text was also included in the Alaska Department of Fish and Game application, but presents justification and analysis of this technique applicable to NMML, ADFG, Oregon Department of Fish and Wildlife, Alaska Sea Life Center, and Oregon State University permit applications for Steller sea lion branding.

A repeated observation of individual animals over their lifetime is precisely the reason for using branding. This is the only currently known way to permanently mark pinnipeds with individual marks that can be easily read from a distance, year after year. As stated in Appendix 1 of our application, the hot-iron branding program conducted by the above groups will provide age-specific survival rates for the eastern and western DPS's (distinct population segment) of Steller sealions, with the ultimate goal of identifying the age and sex of highest mortality, which may facilitate identification of reasons for decline in abundance. Concomitant to this broader goal will be more detailed determinations of metapopulation age-specific survival rates, age-specific reproductive rates, dispersal from natal rookeries by age and sex, site fidelity, and validation of genetic stock dispersal models. As discussed in Appendix 1 of our application, natural marks, flipper tags, and other marking methods are not acceptable for estimating vital rates of Steller sea lion populations.

9. In addition, under the AWA, any time a potentially painful or distressful procedure is to be used, alternatives must be researched and final choices must be justified for the study. In this consideration of alternatives, reduction of numbers, refinement of technique, and replacement with other animals must be considered. If a study exists that requires hot branding (not

considered humane), the length of time the branding takes, post procedure treatment and alleviation of pain, and other options must be considered. In the studies under review, there is no justification for using 4 digits, or for branding each digit alone.

At a minimum, 3 digit branding is sufficient to mark more animals that needed for the studies (34X34X34 – over 39,000 combinations, using only 0 and 1, not "o" and "I"). Additionally, devices should be used that load all three irons together so that only one application of the brand is used (20 sec as opposed to 2 minutes).

PR1: Please explain the need for 4 digit brands, i.e., why aren't 3 digit brands used. Please also explain why each digit is applied separately.

Response: The comment reiterates evaluation criteria that must be considered when choosing and justifying the application of potentially painful or distressful procedure on study animals, all of which were carefully considered in the design of the Steller sea lion survival and vital rate study program. Descriptions of the branding method, including the choices of branding digits, possible effects on the animal, and methods employed to reduce stress, pain and suffering were included in Appendix 1 of our permit application, "Justification for and Summary of Hot-iron Branding on Steller Sea Lions Including Branding on Other Pinnipeds." This text is also applicable to hot-branding proposed in the Oregon Department of Fish and Wildlife, Alaska Sea Life Center, and Oregon State University permit applications for Steller sea lion studies.

To summarize, many different methods of marking pinnipeds have been developed and tested. Hot branding is currently the only known way to permanently mark pinnipeds with codes that can be easily read from a distance, year after year, throughout the animal's life. In coordination with other researchers in the U.S. and Russia, letter designations leading a brand digit sequence specifically designate the natal rookery if branded as a pup. Each individual pup handled at a rookery is marked with that designator followed by an individual sequential number. Choice of digits and combinations included considerations of potential for observer error in recording subsequent observations of a marked sea lion.

If all digits were combined on a single iron, the placement of such a large amount of hot iron to the skin of the animal would concentrate far too much heat in the iron cluster, resulting in an inconsistent transmission of heat and greatly increase the risk of over-branding. Even if the reviewer had suggested a new method of hot branding that avoided this increased risk of over branding, it would not be possible to apply more than one mark (number/letter) at a time. Each digit is carefully applied on a specific spot with extreme care and attention to pressure and time of application. Since the animal's body is not flat, a device designed to place all marks at once would remove the ability to monitor each mark and would result in unacceptable variability in the quality of the mark, and increase a risk of injury to the sea lion. Finally the application of all digits at one time actually works against one of the methods that some researchers have implemented to resolve unreadable brands, purposely "randomly" applying one of the digits off of a straight-horizontal line (either high or low) so that it provides one additional characteristic in the brand photo to compare against future sightings in the event that one of the digits grows/heals to be unreadable. A fixed branding iron would

eliminate this additional criteria used to distinguish individuals, thereby reducing resighting ability and ultimately result in a reduced ability to meet the stated objectives.

10. No consideration has been made for post procedure treatment with antibiotics or pain relief has been addressed. All facilities would be required to have an approved protocol from their IACUC that has shown consideration of alternatives and use of methods that would alleviate discomfort, stress, and long-term complications. Topical antibiotic/anesthetic creme should be used post procedure.

PR1: Please explain what considerations have been made for post-procedure analgesia and antibiotics.

Response: Contrary to the reviewer's comment, a great deal of consideration has been made for post-procedure treatment of Steller sea lions. The narrative below addresses the comments specifically. The NMFS does not have an IACUC for research covered under the MMPA and ESA. However, due to the urging of the Marine Mammal Commission, the NMFS is currently considering developing policy which might require IACUC review for all but observational studies covered by MMPA and ESA Research Permits.

However, this does not mean that the methods used for hot branding have not gone through any IACUC review. Each of the groups requesting branding of Steller sea lions in their permit applications coordinate to follow the same protocols, use similar if not the same equipment and often share personnel and veterinary services. These protocols have been reviewed by the ADF&G Division of Wildlife Conservation IACUC committee and the University of Alaska, Alaska Sea Life Center IACUC committee and these responses come from a coordinated effort between the groups.

The ADF&G Division of Wildlife Conservation IACUC has reviewed and approved the protocols for animal use for this permit application. Alternatives and methods for relieving stress and discomfort were addressed as required. All painful procedures are conducted under general anesthesia. The use of topical anesthetic cream as a post-branding analgesic does not show any promise. Topical anesthetic creams only penetrate 2mm on mucus membranes, are not recommended for wounds, and have a short duration of effect. Topical anesthetic cream are recommended for use on intact skin for procedures such as catheter placement or blood collection. However, the cream must be covered by an occlusive dressing, takes at least 20 to 30 minutes to reach anesthetic effect, the peak of which is at 2 hours (Mathews 2005). In addition, they are generally water soluble, impair healing and would not have any prolonged analgesic effect once the animal entered the water. Thus, they are not indicated for the types of wounds created in the described procedures. Topical antibiotic ointments are <u>contraindicated</u> for deep puncture wounds (as listed on the label) and thus should not be introduced in to biopsy wounds. Topical antibiotic creams include Nolvasan® (which is water soluble and washes off immediately with water) or Silvadene®. Silvadene® is used against bacteria or fungi for human burn patients and could be applied post branding but is normally applied 1 to 2 times daily and kept covered until the wound heals. Thus, applying once, immediately post branding and prior to the port-branding tissue sloughing would probably have a negligible effect and would of course require re-capture of the animal for application

thus making it's use a requirement of additional disturbance.. We do not use antibiotic ointments topical anesthetics in the biopsy wounds as this is contraindicated, contrary to the tenets of wound treatment in veterinary medicine, and increases the likelihood of an anaerobic infection and.

Injectable analgesics have been considered but the risks and short duration of action outweighed the brief post-procedure benefits. Burtorphanol, a narcotic agonist/antagonist could be administered at a dosage used for domestic carnivores of 0.5 to 0.4 mg/kg subcutaneously. This would likely give some analgesia for 6 to 8 hours. However, it does have the risk of CNS or respiratory depression, either of which we want to avoid in a free-ranging, diving mammal and therefore the risk outweigh the benefits. Buprenorphene is another narcotic and has a longer duration of action, 8 to 12 hours. However it is a controlled narcotic (30 times more potent than morphine) and has a higher risk of CNS and respiratory depression than butorphanol and poses an enormous risk for the personnel handling the drug. The non-steroidal anti-inflammatory flunixin meglumine (Banamine®) has been used by marine mammal veterinarians for post-surgical wound pain. It is given at a rate of 1.1 mg/kg IV or IM. The duration of action in pinnipeds is unknown but in carnivores it is given once per day. This is a drug used most often in horses and ruminants, though dogs are highly sensitive to adverse effects on the gastrointestinal lining (such as gastric ulcers) and thus is not approved for use in dogs. A single dose in a pinniped is probably safe but there isn't any published information on the safety or efficacy in these species and therefore its use in this situation is not supported. Meloxicam is another NSAID considered but is it excreted at higher concentrations in milk than in plasma in rats so would not be a good choice for the lactating female. There is no recommended dosage for marine mammals so metabolic scaling would need to be employed to estimate a dose. In reference to the hot-branding of pups, the NSAIDs are not recommended for animals less than 6 weeks of age based on the development of the liver and kidney and should therefore be avoided in rookery aged pups (Mathews 2005). In comparison to the alternatives (i.e. narcotics with respiratory depressive effects), fluninxin meglumine is the best choice we have for juvenile and adults but still carries risks. There isn't a readily apparent safe alternative injectable analgesic for pups on the rookery indicated for extended analgesia post-branding.

Local anesthesia was considered for analgesia post-biopsy and branding. However, a local infusion could be used and for branding would require such a large area would need to be infused and large volume of lidocaine or buprivacaine that the dose could be toxic. If used it would need to be diluted in saline, buffered with sodium bicarbonate and warmed to body temperature. Additionally, infiltration of lidocaine is extremely painful in the human neonate or pediatric patient (Rodriguez and Jordan 2002) and must be administered very slowly. Animals are likely to have a similar hypersensitivity because of their immature peripheral nerves. An inverted L block could be possible but even for the rookery pup, animals would still need to be anesthetized first (because of the extreme pain of the injection) and held much longer under anesthesia for the prolonged injection time and to wait for minimum 10 minutes for the block to take effect putting the rookery pup at higher risk from prolonged gas anesthesia on a mask without intubation. A spinal block would paralyze the swimming ability and a regional nerve block procedure for pinnipeds is unknown (we don't have the landmarks blocked off.

In the case of blubber or muscle biopsies, a deep local block for the biopsy is precluded because the presence of the drug would preclude the purpose of the biopsy. The blubber biopsy is for trace contaminants on the ppb level and stable isotopes. The presence of the anesthetic and its diluents carrier would negate the accuracy of tissue analyses. In the case of the muscle biopsies, these are to be used for histochemistry and electron microscopy, the local anesthetics and their diluents, are not isotonic, and cause cellular disruptions, especially cell swelling, that would negate the point of the biopsy which is to measure muscle cell fiber diameters. Only a regional nerve block away from the site would prevent these disruptions of the cellular matrix. However, a regional nerve block of the nerves to the pectoralis muscles would paralyze the forelimb which we would not consider doing. To do a nerve block to the area of the dorsal biopsy site, the spinal nerve to that area would need to be infiltrated and would likely paralyze the hind flippers. Again, the risk of these local anesthetic procedures out weigh the benefits of the information obtained from the biopsies. Thus, the IACUC considered the minor post-procedure pain levels to be justified. After conducting and observing hundreds of blubber and muscle biopsies on Steller sea lions, harbor seals, elephant seals and fur seals, Dr. Beckmen has not been able to detect any indication from the animals of discomfort at the biopsy sites immediately after recovery from anesthesia. They do not look at the site, try to rub it, lick it or have any perceivable difference in limb movement. We do expect that there should be some discomfort based on our own personal experiences but pinnipeds do not seem to show any overt sign of pain post-biopsy. We have observed that some animals post-branding will scratch their neck (not the area of the brand but that is not easily reached) with their rear flipper. This is usually brief and occurs immediately after recovery.

Mathews K A. 2005. Analgesia for the pregnant, lactating and neonatal to pediatric cat and dog. J Vet Emergency Critical Care. 15(4): 273-284.

Rodriguez E and Jordan R. 2002. Contemporary trends in pediatric sedation and analgesia. Pediatric emergency medicine: current concepts and controversies. Emergency Med Clin North Am. 1:199-222.

11. No consideration was given to proper recovery times for anesthesia. While an animal may be awake and mobile within 20 minutes of cessation of isoflurane, it is a recognized occurrence in veterinary medicine that the effects of anesthesia do not dissipate after 20 minutes. In practice, we have seen animals take up to 24-48 hours to recover from anesthesia, especially when the procedure of lengthy (over an hour).

PR1: Please explain what considerations are made for recovery of animals from anesthesia versus the initial return to consciousness.

Response: Although we hold animals over 1 hour, they are never under isoflurane gas for that long. Sealions are kept on oxygen for 5 to 10 minutes after the isoflurane is discontinued. They are then moved to the recovery area and allowed to breathe ambient air. The anesthetist remains in immediate contact with the animal until it is extubated. After extubation, the anesthetist observes the recovery until the animal is conscious. The animal is allowed to remain in the recovery area as long as it wants and remains under observation by at least one

biologist until it departs. The animal must be fully recovered, very alert and ambulate normally before they leave the recovery area. A physical barrier is used to prevent premature departure until full recover is achieved. Consciousness alone is not criteria for release; they must be alert, responsive and maintaining normal postures. The vast majorities of sea lions are fully alert and react normally with a flight response and swim/dive within 20 minutes of the cessation of isoflurane but not all are allowed to depart or try to depart that quickly. If the animal has had a problem, such as a fall during capture or an anesthetic complication, then they are held in a recovery cage or stall until the veterinarian judges the animal is ready for release. Animals have been held over 12 hours as a precaution. The commenter does not specify what type of anesthetic was used when they observed a recovery time of 24-48 hours, nor what species they are referring to. In our experience, there has not seemed to be any prolonged recovery from isoflurane and the maximum time of any detectable behavioral sign sedation when isoflurane has been used alone, is less than 1 hour.

12. While small surface skin biopsies may be acceptable without anesthesia under some conditions, proposals for blubber and muscle biopsies, some up to 2 ½ inches deep, constitute painful and invasive procedures and must be done under anesthesia. If local anesthesia is to be used, dosages should be given, and well as documented protocols for determination of effectiveness, including the waiting period for full effect.

PR1: Please provide details about use of anesthesia for blubber and muscle biopsy, including dosages, how you determined the specific anesthetic/dosage would provide appropriate level of anesthesia, and how long you would wait post-deliver for the anesthetic to take effect. If you do not intend to use anesthesia for these procedures, please explain why.

Response: The NMML does not request authorization for muscle biopsies in this application. They have been performed in the past under general anesthesia at a surgical plane of analgesia. Likewise, in recent practice all blubber biopsies have been conducted under general anesthesia at a surgical plane of analgesia. On page 22 of our application we describe the use of Lidocaine as a local anesthetic should we remove a blubber biopsy from an animal that is physically restrained.

13. The amount of blood expected to be taken at sampling, while below 10% of blood volume, are significantly high. Given the state of current laboratory methods, it seems that samples can be much smaller, as most tests no longer require 5 ml of serum anymore, more like 0.1 ml. Remember refinement and reduction – this can be applied to sampling as well.

PR1: Please explain why your specific studies require collection of the amount of blood requested in your application.

Response: This comment incorrectly characterizes our requested sampling volume, volumes required for laboratory analyses, and ignores unique characteristics of the pinniped circulatory system. Reducing blood sampling volumes to a minimum required to achieve analytical and archival (for future research to provide baseline data) needs is among the critical considerations to ensure animal welfare, and we have indeed done so appropriately.

Pinnipeds have up to two times the blood volume of terrestrial mammals, and is 90-100 mL/kg for Steller sea lions <30 months old, and about 120 mL/kg for adult females (Richmond et al. 2006). Thus our requested sampling scheme of drawing 1 mL blood/animal kg is a proportion of about 1% of the blood volume of pups-juveniles, and 0.8% of the total blood volume of adult females, an order of magnitude less than a 10% sampling volume. These are extremely conservative sampling rates. By comparison, ppediatric human phlebotomy guidelines recommend up to approximately 1.2 mL/kg as a maximum volume to be withdrawn at any one time (Garza and Becan-McBride 1984). Finally, our sampling requirements are determined by volumes required for analyses as directed by the analytical or research laboratories analyzing the samples. While some analyses may require small amounts of plasma or serum, non-clinical analyses are typically run in duplicate or triplicate, and because pinnipeds have much higher packed cell volumes than terrestrial mammals the expansion to the whole blood volume that must be collected to result in sufficient plasma or serum is approximately double. Thus, our sampling requirements range from 1 mL whole blood for analyses of leptin for haptoglobin levels, through up to 16 mL total withdrawal for deuterated water determination of body composition.

Garza, D. and K. Becan-McBride. 1984. Phlebotomy handbook. Prentice-Hall, CT. 305 p.

14. As discussed above, all should address the potentially painful procedures and care of the animals.

PR1: Please explain measures that would be taken during and after potentially painful procedures.

Response: It is not clear from this comment exactly what are being referred to as "painful procedures." However, as noted in our response to comment number 10 virtually all procedures involving sample collection similar to those conducted during domestic veterinary treatments are conducted under gas anesthesia in a surgical plane, and there is no pain to an animal under that condition. The attending veterinarian is responsible for weighting the risks imparted by anesthesia versus any temporary discomfort from a procedure.

15. Applicants identify that studies have already proven that certain methods are equivalent to the "gold standard" –deuterium(?) measures – for determining body composition. Therefore, it appears to be redundant and unnecessary for more than one method be used, increasing the handling and sedation/anesthesia. Only one method for determination of body composition should be used for each study. The intent of the studies is to monitor the animals, not compare methodologies. As an endangered species, these sea lions should be studied, not experimented on.

PR1: Please explain why it is necessary to use multiple methods on the same animal for determining body composition and why comparison of the methods cannot be performed using a non-ESA listed species.

Response: We requested authorization for three procedures that assess body condition. Measuring the dilution of labeled (deuterated) water injected in to an animal allows estimation

of the total body lipid stores, yet requires holding times of up to 2.5 hours to allow distribution of the labeled water throughout the total body water pool. In contrast, BIA may be an acceptable alternative and passively measures electric resistance of a body, which is related to body water (and thus body lipid) content. The evaluation of this technique must be made on a species-specific basis, and much of the data for that comparison have been collected and are currently being analyzed. The BIA procedure is by contrast a very short duration and minimally invasive procedure that if proves to be an acceptable alternative to deuterated water injection would greatly shorten holding times required. Currently the BIA measurement can provide a lower-resolution back-up upon which to estimate body condition should the deuterium dilution method not be successful in an individual. In either case though, we need to retain authority to do either procedure. A third procedure we utilize is external ultrasonographic determination of blubber thickness. Neither deuterated water nor BIA provides information on blubber thickness, nor does ultrasound provide information on whole body lipid content, so the techniques are not redundant.

16. The application should include specifics, not generalities. Specific sites, animal numbers, etc., need to be identified.

Response: The comment is correct that the NMML application did not provide specifics of activities involving resights, scat collection or juvenile/adult capture activities. However, the application was based on the general projection of overall impact. Logistics and budget dictate specific sites visited within any year, and those plans are created annually. Scat collections are associated with the rookery activities detailed in Tables 2-4, and with the location of brand resighting activity. Resighting will occur at any time we are in the field regardless of the purpose, but specifically target haulouts and rookeries throughout the central-eastern Aleutian Islands and the western-eastern gulf of Alaska sites listed in Table 1. The ability to survey specific areas for resights is ultimately determined by the presence or absence of sea lions on the site and the environmental conditions that may limit the work. These constraints result in some generalities used to describe the work because it is simply impossible to know exactly what site would be visited at what exact time. This represents the broad study area, specifics are determined in as much detail as possible each year in collaboration with other investigators conducting research in the affected areas. Annual plans with survey routes provided to AKR early each year, and are updated throughout the year. Annual plans are shared and modified with other investigators either informally or formally at coordination meetings.

17. The general comments above apply to this application as well. Since there is no IACUC in place yet, detailed information is needed so that your office can adequately review. Justification of the studies should not be predicated on a prior study, but must be valid studies in their own right.

Response: Our initial permit application submitted on December 1, 2006 was reviewed by the NMFS Office of Protected Resources Permits Division (PR1). NMFS PR1 requested we provide additional information and clarifications on December 22, 2006. We submitted a revised application responding to NMFS PR1 comments on January 12, 2007. An acknowledging electronic mail sent from PR1 on January 16, 2007 did not request any

additional information nor note remaining deficiencies.

18. Tooth extraction (this can be applied to all applications as well) has not been justified, as there are no aspects of the study that require precise aging. The categories referenced are broad and the experienced and trained researchers should be able to tell approximate age without having to pull a tooth and the dangers the procedure entails (anesthesia, malocclusion, dental abscesses, pain).

Response: It is incorrect to state that no aspects of the study require precise aging. One of the fundamental needs to understanding the reasons for the decline of Steller sea lions is the ability to recognize whether a threat is specific to a particular cohort or age class. There are currently only two methods of reliable aging in Steller sea lions: 1) aging by counting the incremental growth layers on a longitudinal section or 2) by permanently marking the animal at a known age. One of the most useful measures of body condition is the mass or size of a sea lion relative to its age. Linking data collected on foraging behavior, health and condition to a known-age has and continues to be a critical component of our studies. Past evidence for decreased body condition was based on size-at-age, and long-term and spatial comparisons depend upon accurate estimates of age. As juveniles were considered to be an age-group most vulnerable to shifts in prey abundance, the development of their physiological and behavioral capabilities has been a central focus. The ages of pups and juveniles captured under the current permit are estimated based on a combination of teeth eruption pattern, general animal size, and season of capture. This technique becomes much less precise after a sea lion is older than one year. Based on King et al. (2007) it appears that tooth extraction may no longer be necessary for animals <2 years old to determine age and the necessity for this procedure has been reduced.

King JC, TS Gelatt, KW Pitcher, and GW Pendleton. 2007. A field-based method for estimating age in free-ranging Steller sea lions (*Eumetopias jubatus*) less than twenty-four months of age. Mar Mammal Sci 23(2):262-271.

19. Any decision on the use of anesthesia needs to be left to the veterinarian, not the PI, unless he/she is a qualified veterinarian.

Response: All protocols for sedation and anesthesia and analgesia are created in collaboration with qualified marine mammal veterinarians.

The Humane Society of the US

20. Though Table 2 provides latitude and longitude for a variety of rookeries and haul outs, the summary chart (Table 1) generally states that sampling is "west of 144° W, AK" or "WA, OR and CA." This is not helpful in demonstrating that sampling is systematic, robust and non-duplicative in nature. This concern is magnified by some of the text asserting that there is greater concern with some areas than others with regard to the ongoing declines evident in portions of the range of Western DPS Steller sea lions.

Response: Tables 2-4 of our application explicitly state locations associated with aerial

surveys and pup handling activities. Also see response to comment number 16.

21. We agree with the priority given to recommendations of previous work groups for additional study in key areas, however questions such as survivorship can effectively be addressed only with considerable re-sighting effort. There is no discussion of the proportion of effort dedicated to resighting previously marked animals nor is there consideration that a one-time sampling of an animal's body condition in service of investigating the nutritional stress hypothesis can only provide a snapshot of condition at a single point in time whereas understanding effects may require a more longitudinal study (e.g., re-capture and sampling of previously branded animals to monitor changes over time). We see no specific mention of resighting activity either independently or as part of other activities listed under the paragraph labeled section "b. Narrative Account of Research." Given the focus on juvenile survival and female fecundity that the applicant states are necessary under Activity 6, it would be helpful to have an explanation of plans for resighting effort.

Response: Only general areas for resight effort were presented in the application to represent the maximum potential takes associated with this activity each year. Due to weather, sea lion behavior, and funding it is not possible to specifically state which sites will be observed in any given year. However, annual study plans are developed and coordinated with other researchers (as described in the DEIS to ensure adequate coverage and minimize disturbance. Detailed descriptions were not included in the permit application, however, this activity is among the most important, if not the most important, of studies we conduct.

To estimate vital rates of Steller sea lions, NMML uses Cormack-Jolly-Seber (CJS) mark-recapture models, which have been packaged within the Program MARK by Gary White at Colorado State University[1]. These models require the release of individually marked animals into the wild population and individual sightings histories (recaptures) over many years. To comport with the model assumptions, NMML conducts sighting effort each year during the summer (May through August) at approximate annual intervals following the date of initial marking as pups in June and July.

NMML collects observations and photographs of marked animals each year both from land-based and skiff-based observers. Land-based observations primarily come from two islands with rookeries where pups have been branded every other year since 2000: Marmot and Ugamak Islands. Researchers are stationed on these islands at field camps for 2+ months from late May through early August each year and collect a wide variety of information of sea lion attendance, behavior, and observations of marked animals from cliff-side observations posts high above the rookeries. Land-based observations from field camp-based researchers are conducted each day with no disturbance to the animals, since they are unaware of the presence of researchers.

NMML also conducts brand-sighting cruises during which researchers observe sea lions from small skiffs just offshore of haulouts and rookeries in the Gulf of Alaska and Aleutian Islands. During these cruises, researchers may also go ashore in some locations to observe sea lions

[1] http://www.warnercnr.colostate.edu/~gwhite/mark/mark.htm

from vantage points that are preferable to those available from the skiff. Sea lions are aware of the presence of researchers during skiff-based observations, and most will become alert, look at the researchers and vocalize. Researchers try to avoid large disturbances, such as those that might cause them to enter the water, since this would preclude further effort to record sightings and photograph marked animals. Researchers may (weather and time permitting) visit sites with large numbers of sea lions on multiple occasions, sometimes on successive days. This is because groups of sea lions that use a particular site may be at-sea during the first day of observation and haul-out on the second.

NMML also receives information about sightings of Steller sea lions marked by NMML from other research organizations (e.g., Alaska SeaLife Center, Alaska Department of Fish and Game), as well as native Alaskan organizations (e.g., The Alaska Sea Otter and Steller Sea Lion Commission) and members of the public.

For analyses of survivorship, NMML collects the following data individually for each branded Steller sea lion observed: date and time of observation, location of observation, brand letter and/or numbers, assessment of brand quality, estimate of age (young of year, juvenile, adult), estimate of sex (if juvenile), animal behavior and general comments. Individual impressions of brand numbers were recorded prior to group discussions among all observers, and then discussed to develop a consensus opinion either on site or later back on the ship or at camp while reviewing digital photographic images. Observations continue until observers were satisfied that the left sides (where brands have been applied since 2000) of the vast majority of sea lions had been observed. On vessel-based surveys, numbers of sea lions disturbed by activities (defined as those that were flushed into the water as a result of counting, resighting, or scat collection) were also recorded. Information on weather and sea state is also recorded, along with a count of all animals on the haulout or rookery. The CJS models also estimate the sighting probability based on the patterns within the sighting histories of each individually marked animal.

To estimate natality of adult females, not only must we have collected information on their survival, but we must determine whether each marked adult female has given birth to a pup. Land-based observers may observe marked females giving birth. Later in the summer, births can also be verified by observing a marked female nursing a new pup. However, a suckling bout has its own probability of detection which is in addition to the probability of observing the marked female herself. To estimate this probability, NMML is using a methodology developed by K. Hastings (Alaska Department of Fish and Game) in which the behaviors of marked females and the associations of them with nearby pups are recorded during multiple, brief (~10 minute) periods of observation. Data collected during each observation period include standard information on effort (date, time, count of animals, weather) and on the marked female (described above with respect to survival analyses). In addition, information on the reproductive status of the marked female is obtained by recording the 'highest' reproductive behavior observed during each observation bout (reproductive status codes listed from 'lowest' to 'highest' indication that the marked female gave birth during the year):

a. Marked female is alone (no pup nearby)
b. Marked female laying beside or touching a pup, with no interaction observed; isolated pair in larger group

c. Marked female laying beside or touching a pup, with no interaction observed; isolated pair distant from other females
d. Marked female-pup pair interacts significantly: sniffing, touching for < 3 min; and no sign of rejection/aggression towards the pup is observed
e. Marked female-pup pair interacts significantly for > 3 min, pair moves around rookery together (distinct from movement of other animals in area) and remain in contact afterwards, or pair (physically distant from each other) reunite after calling (e.g., touching nose and sniffing) and remain together afterwards
f. Pup laying on top of marked female
g. Marked female nurses pup briefly; limited observation
h. Marked female nurses pup; extended observation

This same hierarchical list of behaviors is also used to characterize marked adult female interactions with juvenile sea lions.

22. We note that NMML states that it will notify the regional office at least one month prior to field work. However other applicants (e.g., see comments on Horning above) have stated that they plan to determine sampling areas several months in advance, making it difficult for this applicant to avoid or help others avoid duplicate sampling or unnecessary disturbance.

Response: That text in our permit application simply restated a standard notification condition included in all Steller sea lion research permits, and the associated text presented on page 7 of our application only generally describes the coordination and planning efforts for Steller sea lion research. Additional description of coordination is found in the final EIS page 3-37 (3.2.1.12 Coordination of Research). Sampling areas are determined several months in advance, and researchers meet annually (formally) to coordinate plans, typically by February, but continue to coordinate throughout the year informally.

23. The applicant proposes both aerial surveys and drive counts of animals to collect data for abundance and trends in population. The NMML never explains why both are deemed necessary. Indeed other applicants (see Wynn above) only use photogrammetry from aerial surveys. This applicant should be limited to the use of that less risk prone method.

Response: NMFS uses aerial surveys to monitor population trends of non-pups at haulouts and rookeries. Prior to 2001 NMFS solely utilized ground-based counts to estimate pup abundance, but reliance on this technique has been decreasing. In 2001, pup counts were supplemented with counts from aerial photographs at three sites that ship-based personnel were unable to count (Sease and Gudmundson 2002). An analysis that compared aerial and ground-based pup counts at 16 western stock rookery and haul-out sites among four years of surveys by Fritz and Stinchomb (2005) suggested that medium-format (MF) aerial photogrammetry could be used to index pup counts consistent with beach counts. This led to the first Alaska-wide MF aerial pup survey being conducted in 2005, which was supplemented by ground counts at two sites that were not photographed due to weather. This study also provided comparison of 19 sites counted by both techniques, and suggested only a few sites where counts did not agree, perhaps due to topography. Biennial pup count surveys will now principally rely on aerial MF photogrammetry, but to ensure a complete abundance

estimate requires authorization to supplement with ground-based counts, if needed, at sites not amenable to aerial surveys or that are missed due to weather.

Fritz, LW and C Stinchcomb. 2005. Aerial, ship, and land-based surveys of Steller sea lions (*Eumetopias jubatus*) in the western stock in Alaska, June and July 2003 and 2004. U.S. Dep. Commer., NOAA Tech Memo. NMFS-AFSC-153, 56 p.

Sease, JL and C J Gudmundson. 2002. Aerial and land-based surveys of Steller sea lions (*Eumetopias jubatus*) from the western stock in Alaska, June and July 2001 and 2002. U. S. Dep. Commer., NOAA Tech. Memo. NMFS-AFSC-131, 45 p.

24. The applicants propose to capture from the Western DPS, 1100 pups (5-days to 2 months old) and 120 juveniles (2 months through 3 years) and 60 adults (over 3 years). We wish to note that the DEIS classified only pups and non-pups. Non-pups were defined as animals over 3 months of age. Given that this applicant and others use categories for sampling that are different than those in the DEIS, we are not clear how takes will be reported relative to understanding whether impacts are within the estimates used/approved by the DEIS.

Response: NMML requested capture takes to the finest age class possible depending upon study objectives and time of year, and other investigators do likewise. We report either the exact age of a captured animal if determined or as the appropriate age class categorization from our requested takes in Table 1 of our application. Because of different objectives, seasons of capture, and age groupings presented by multiple investigators, the EIS analysis lumped age categories for analyses. This process is described in the final EIS on pages 4-28 and 4-29.

25. The summary chart accompanying the application (Table 1) inappropriately lumps all activities related to captures including "physical or chemical" restraint. It is important to know which animals will receive anesthesia and which will not. Anesthesia carries some attendant risks but also some clear benefit (analgesia and sedative) and the degree of its use should be clear in the summary chart. In fact, the text indicates that it will not always be used. The application states that pups are provided gas anesthesia if they will be branded to "reduce stress on pups." Apparently there is little concern for adults. The applicant states in its discussion of adult captures that the "squeeze cage…restricts movement without the need for immobilizing drugs." In its discussion of branding juveniles, the application states that sedation will be provided "if appropriate at the discretion of the attending principal investigator or veterinarian." This seems inappropriate. Given the statements about the pain of branding in the DEIS and the commitment to its use by applicants such as Horning (not to mention its discussion by applicant Trites who proposes to study pain response in branded and non-branded animals) anesthesia should be used if research is to be humane. 16 U.S.C. § 1374(b)(2)(B); 50 C.F.R. § 216.34(a)(1) (research must be humane).

Response: NMML has requested to apply brands to anesthetized Steller sea lions only regardless of age, and this procedure is explicitly stated in two separate parts of our permit application. In the research narrative on page 23 we describe that pups (<2 months old) are first anesthetized with isoflurane gas prior to brand application, and state on page 24 that: "Sea lions >2 months old (if not previously branded as pups) are branded under anesthesia

following the same protocols." This procedure is also reiterated in our description of measures to minimize effects on page 35: "Hot-brands are applied only when an animal is under general anesthesia." Similarly, the use of anesthesia (general or local, as appropriate) for procedures involving the likelihood of more than transitory pain or discomfort is described under each sampling activity on pages 21-26 of our application. The commenter may be referring to the observation that at times with large animals that are difficult to handle, the attending veterinarian may recommend that some sedative like valium be used to calm the animal so that it can be safely anesthetized with isoflurane gas. In other cases when procedures such as hot-branding are not employed full anesthesia by gas becomes unnecessarily risky. We would argue that it is indeed appropriate for the attending veterinarian to determine if this sedation is necessary rather than to automatically sedate all animals at the time of capture regardless of their disposition. The action recommended by the comment seems inappropriate, unsubstantiated by any veterinary protocol, and unnecessarily dangerous.

26. This application provides assurances that branding is not likely to lead to direct or indirect mortality, citing a study at Ugamak Island (section D). This fails to mention results of an Oregon study that is referenced earlier in the text. Further, we do not see plans for extended monitoring of animals to ascertain their fate, as occurred in Oregon.

Response: This comment is related to comments number 28 and 29. We present below a response after comment number 29 addressing all three comments.

27. The application states that a number of procedures will be performed on captured sea lions (e.g., blood collection, blubber biopsy, fecal loops, tooth extraction, bioelectrical impedance analysis pulling vibrissae, attachment of scientific instruments, etc) However, the applicant states that "criteria for each procedure will be dependent on the specific study objectives *at the time of capture*." The point of a research application is to specify the study objectives in advance and enumerate the procedures that are necessary to fulfill its goals. It is inappropriate to prevent analysis of impacts of a permit by failing to specify the "criteria for each procedure" in advance.

Response: Table 1 in our permit application states the maximum number of takes associated with procedures that may be performed on captured sea lions each year, and can be used to assess the maximum potential impact of proposed activities. The referenced statement from our application was simply meant to imply that it is not always appropriate or necessary to subject each captured animal to each procedure. Nor depending on funding, logistics, availability of appropriately experienced personnel, or changing priorities will each procedure necessarily be performed. However, because a permit modification can take more than a year to process, it is necessary to maintain maximum flexibility in authorized activities, so long as these activities are consistent with overall study objectives and the maximum potential impact, should all take authorizations be exercised, can be evaluated.

28. NMML has proposed to attach VHF transmitters to pups as young as 5 days of age (paragraph (i)). This seems inappropriately risk prone. The previous paragraph (h) on branding stated that pups older than 2 weeks are selected for post-natal survival studies so it is not clear why it is necessary to instrument pups this young. There appears no need to impair movement

and risk mother-pup bonding of pups as young as 5-14 days of age by attaching hard antennaed instruments.

Response: This comment is related to comments number 26 and 29. We present below a response after comment number 29 addressing all three comments.

29. Although the applicants cite a study done in Oregon to determine post-branding survival, they did not provide results, which showed that branded pups appeared to be adversely affected. While it may be important to replicate such a study, we see no mention made of where or how researchers will return to the site. The Oregon study (Scordino 2006) was a more traveled area (dead pups were reported by fishermen in some cases) and researchers returned multiple times to the site. If the applicant plans to do this, it is not clear in the permit application. If they do not plan to do this, it may impair the results of the study.

Response: This activity was included in response to public comments as a possible method to broaden post-handling monitoring efforts. The comments correctly point out that many factors must be considered if such a study is to be a useful monitoring tool at Alaskan sites, but misstate potential effects. This study would be conducted on a sub-set of pups chosen for branding at locations with field camps already established to monitor sea lion biology and research effects, and thus no additional risk of mother-pup bonding is incurred beyond that associated with the scheduled rookery visit for branding. Telemetry instruments may be VHF, RFID, or some other technology, but in none of the instances utilize "hard antennae".

The Oregon study is a Master's thesis of a graduate student and not a peer-reviewed scientific analysis and presentation. The data collected during the Scordino thesis project are still being analyzed in conjunction with data collected after the thesis project ended, and the thesis clearly stated in the conclusions that all estimates were based on "apparent survival" and confounded by emigration. Studies on Ugamak Island and Lowrie Island have not indicated similar effects.

30. In this permit, the section on Effects on Stocks discusses the effects of anesthesia. It fails to include some of the caveats outlined in the DEIS (e.g., lack of reversal agents) and cites a study involving the use of darting (included as appendix Table 7) concluding that only 1.9% of sub-adult animals that "remained observable" died. But the Table indicates that of the 72 darted animals, 28 went into the water or to an inaccessible location, where their condition could not be monitored. It is disingenuous to state that only 1 died when the status of 28 could not be observed after darting. Further two of only 16 juveniles darted was observed dead, with 9 of the 16 unobservable. This procedure should not be allowed. . 50 C.F.R. § 216.34(a)(1) (the applicant must demonstrate the that activity is "humane and does not present any unnecessary risks to the health" of the animal).

Response: This mortality rate was estimated based on sea lions that were observed and thus had known fates, at least within the time period of observation. An estimate can not be determined from no data. However, this analysis assumed that the unobserved animals responded in the same manner as the observed animals.

The Draft Steller Sea Lion Recovery Plan explicitly states that one of the most important age classes to investigate are adult animals, specifically adult females. To date the only method of capturing those animals on a haulout or rookery without some sort of fixed cage is by the use of injectable anesthetics. Although every possible measure is taken to ensure that this would not result in a lethal take it is recognized that there is always the risk. This is among the reasons that lethal takes incidental to the research activities are requested in the permit application.

31. We note that the applicants wish to use deuterated water. This was not discussed or analyzed for impact in the DEIS and should not be permitted. The applicant has linked its use with the use of bioelectrical impedance analysis (BIA). If the effective use of this procedure requires, as stated, "a mathematical relationship between values from BIA and other measures such as deuterated water" then subjecting animals to subcutaneous needles required by BIA may also be inappropriate or unnecessary. Unanalyzed procedures should not be allowed.

Response: See response to comment number 15 regarding the use of BIA and deuterium. The use of deuterium is discussed in the final EIS in Appendix B 2.11, and in the effects analysis of low-risk procedures on page 4-33.

32. A number of the proposed procedures are slated for use only in Western DPS Steller sea lions (e.g., stomach tubes, enemas, BIA, ultrasonic imaging) and not Eastern stock. Other procedures are used on both (e.g. blubber biopsy, fecal loops, pulling vibrissae). This is not explained but should be.

Response: Steller sea lion captures in Washington occur incidental to a California sea lion capture program, and some, but not all of the procedures performed in Alaska are part of the study design for animals handled in Washington. Reducing the number of procedures performed on an animal is a responsible consideration to ensure animal welfare.

33. The discussion of mortalities appears limited. See 50 C.F.R. § 216.34(a)(7) (NMFS may not issue a permit if the requested action will "likely result in the taking of marine mammals . . . beyond those authorized by the permit). Assertions of low levels of past incidental mortalities across all permits does not include a number of mortalities provided by NMFS in documents submitted to U.S. District Court as part of litigation on Steller sea lion permit issuance in 2005. Further, the applicant cites a 2002 EA concluding that the amount of accidental mortality would not have a significant impact on the stock. This was the same conclusion of the 2005 EA that was found inadequate in its analysis by a U.S. District Court judge. Humane Soc. of the U.S. v. DOC, 432 F. Supp. 2d 4 (D.D.C. 2006). This further highlights why the regulations require that a Final EIS be available to the public during the comment period, so the commenters be informed as to true environmental impact of the research permits. 50 C.F.R. § 213.33(d)(iv). After requesting an allowable incidental mortality of 5 Western DPS Steller sea lions per year in C.4, the applicants also state that they "expect that this number may be modified by the permit office during the permit application and evaluation process." The meaning of this sentence is unclear.

Response: Mortality discussions in the NMML permit application present information relevant to the requested activities. A broader treatment of the potential for research-related

mortalities and impact on Steller sea lion stocks can be found in the final EIS. The meaning of the quoted sentence was simply that NMML expected that the NMFS permit office would ultimately set mortality limits for all Steller sea lion research permits as was done in 2002, regardless of requested takes.

34. Although the applicants request 5 mortalities from the Western stock in its text, the summary chart (Table 1, page 45) states that 1 mortality is expected from the Eastern DPS and 10 mortalities are expected each year in the Western DPS, with an asterisk stating that it is not to exceed 5 in the Western stock. This is confusing to say the least and should be clarified.

Response: This confusion arises from an editorial error on Table 1 of our permit application conflicting with the request correctly stated in the text. The footnote read correctly that the number would not exceed 5 in the western stock. A corrected Table 1, Activity 5 should only show a request for 5 mortalities per year among the western stock, and 1 mortality per year among the eastern stock.

35. The applicants state that capture related myopathy has not been observed in pinnipeds. This is a meaningless assurance. First, by the DEIS' own admission, there has been virtually no study of effects of intrusive capture and sampling studies, so it is disingenuous to presume that it is not a very real risk. Further, the cause of the documented deaths of branded animals found well after branding (e.g., in Scordino's study) has generally not been determined, but post-capture myopathy cannot be ruled out. There is every reason to believe that this phenomenon occurs in pinnipeds, as it has certainly been raised as a concern for both terrestrial and marine mammals that have been studied. The reference in the DEIS for deaths from capture myopathy (Fowler 1986) is from a report of a workshop on the status of northern fur seals and research. Bottlenose dolphins are at risk from capture myopathy (Colgrove, 1978) and it is of sufficient concern to stranded marine mammals of multiple species that it is addressed in the DPEIS for the marine mammal standing and health network. (NMFS 2007) There are myriad publications discussing this phenomenon in a huge array of taxa in which capture myopathy has resulted from transport, stress and struggle. (e.g., EFSA, 2004; NMFS/SWFSC; CCAC, 1984). It is inappropriate to discount it for Steller sea lions.

Response: This statement was taken out of context from our application. While the comment is correct that the statement in our application says capture myopathies have not been observed in pinnipeds, it goes on to state that regardless of that lack of an observation, we assume that capture events are indeed stressful for Steller sea lions. Thus it is appropriate to incorporate post-release monitoring to the extent practicable to assess whether any post-handling mortality occurs, regardless of potential mechanisms.

36. The section in the application dealing with NEPA compliance states that NMFS does not have an IACUC under which research needs to be approved to guarantee compliance with the AWA. But it should.

Response: While this comment is related to our application, we suggest a response discussing the relationship between NMFS and the AWA and IACUC implementation would be more appropriate from headquarters. We note that NMFS is currently in a review process

evaluating implementation of IACUC procedures for agency marine mammal studies. This process is described in Chapter 5 of the final EIS.

37. Much that is proposed under this permit involves the use of novel capture techniques, the use of protocols not assessed in the DEIS, targeting age classes or sexes not differentiated in the DEIS and the use of techniques that arguably do not comply with the MMPA strictures on humane research (e.g., branding without anesthesia, use of duplicative drive counts when aerial photogrammetry is available, etc.) Errors and omissions need to be corrected and procedures and analyses should be consistent with those in the DEIS. Because this permit relies on procedures not in the DEIS, uses more invasive measures when less invasive procedures are available and relies for its understanding of the impact of invasive procedures on the somewhat arbitrary impact analyses in the DEIS, this permit should not be granted at this time.

Response: We disagree that the permit should not be permitted, and all of the points raised in this comment specifically regarding our application have been addressed in the above responses. The remainder of this comment addresses issues related to the NEPA process and the EIS, not to our permit application, and as such a response from PR1 may be more appropriate. However, we would note that procedures for analyzing and permitting new applications and study methods are discussed in Chapter 5 of the final EIS.

Figure 1. Steller sea lions associated with a pen capture area at Kodiak Island, Alaska. Photo credit: Dr. Jane McKenzie, University of Alaska.

Response to Public comments received on File No. 358-1888 (Alaska Dept. Fish & Game, Steller sea lion research) compiled by Dr. Lorrie Rea and Dr. Kimberlee Beckmen (ADFG Wildlife Veterinarian). Some responses have been developed in collaboration with NMML and other permit applicants who have received similar public comment.

Marine Mammal Commission (MMC)

1. The application on page 14 states that newborns will be branded, but on page 23 states that pups under 20 kg or with an umbilicus will not be branded. This apparent inconsistency needs to be resolved;

Response: This apparent inconsistency stems from our undefined use of the term newborn, which we consider to be pups within days to weeks of birth that do not yet enter the water. Animals which are less than 20 kg or have a visible umbilicus attached are a subset of these newborn animals which we do not intend to brand as outlined later in the paragraph on page 14 as the exception to the general request. These animals will be tagged instead of branded so that they are not recaptured and restrained again during the operation. We have also outlined this exception on page 21 (which I believe was referred to as page 23 since there is no mention of branding on page 23).

2. Page 33 of the application states that "ADFG had 2 juvenile mortalities occur during a capture trip in 2004 and 15 pups die during branding operations." However, Table 2b on page 69 does not reflect this information. Information concerning the total number of animals, by age group, that died during research activities, the circumstances surrounding those mortalities, and what steps, if any, are proposed to reduce the number of mortalities during future research needs to be provided;

Response: Table 2b provided in Appendix 1 on the effects of pup branding operations was originally compiled to analyze operations from 2000 to 2004 by all agencies branding at that time. It is noted in this table that ADFG had experienced 14 pup mortalities in Southeast Alaska during operations between 2000 and 2003. The 15th pup mortality noted on page 28 of our application (referenced as page 33 in the public comment) occurred on 24 June 2005 as a result of a poor recovery from the anesthesia and is reported in detail in our annual permit report titled "Report of activities on Steller sea lions for 2005-2006: MMPA and ESA Permit No. 358-1769-00." The two juvenile mortalities noted in 2004 did not occur during pup branding operations and thus were not included in Table 2b., however a complete description of circumstances and necropsy reports for these mortalities are included in our annual permit report titled "Report of Activities on Steller sea lions for 2004: MMPA and ESA permit 358-1564 (amendments 06-07)". One of these animals (11 month old pup captured by net in Aleutian Islands on 7 May 2007) had its airway impacted by a bad position in the capture net during restraint, and the other (23 month old in the Aleutians on 13May 2004) aspirated stomach contents while under anesthesia and could not be revived. In these annual reports we outlined our proposed actions to reduce the number of mortalities during future research, and summarizing from the 2005-2006 report below:

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

In all of our operations, both branding of pups and capture and study of juveniles, gas anesthesia has been used to mitigate any pain or stress associated with application of permanent marks and the handling and collection of body measurements and physiological samples. There have been isolated accidental deaths associated with the anesthesia process, but these numbers have been very low in comparison to the total number of animals handled. One of these mortalities has been associated with equipment

malfunction, and during the inspection of the equipment by an outside expert it was determined to be caused by a rare manufacturer's defect." In some cases no level of mitigation will guarantee that an animal will not react in an unpredictable and negative way to anesthesia (as is true in all vertebrates, including man). All anesthesia is performed by trained veterinary staff, and their sole responsibility is the care of the animal. On many trips we have multiple veterinarians present, and our ADFG staff are also trained to watch for any indication of poor condition of animals while they are in our care. The Aleutian pup mortality resulting from the net capture event, resulted in a thorough review of our capture techniques and equipment and led to the modification of our protocols to enable ensure that animals would not be held in nets for longer periods of time than absolutely necessary, especially when working in rugged terrain where posture of the animal could not be adjusted easily.

An overview of research related mortalities for all permit holders has been presented in the PEIS beginning on page 3-26 in section 3.2.1.6 Anthropogenic sources with further detail provided in section 4.8.1.

3. The application does not, but should, discuss the potential effects of Telazol and other proposed drugs on the nursing pups of females on which the drug(s) will be administered. Also, because of a risk of mortality, both from the drug and from drowning if animals enter the water before the drug takes full effect, the Commission believes that every precaution should be taken when using these drugs and that only veterinarians and biologists with significant experience in darting marine mammals be authorized to conduct activities involving their use;

Response: The excretion of Telazol® in milk has not been specifically studied but the two drugs that comprise the solution; tiletamine and zolazepam which belong to drug classes, cylohexamines and benzodiazapines, respectively, the latter of which has been studied. In humans, when infants nursed on mothers shortly after high dose administration of diazepam (a benzodiazepine), the plasma concentration of the drug in the infant was less than 10% of that of the mother and was well below the concentration that could produce a clinical effect (i.e. sedation) or complication (Hale 1999; Lee and Rubin 1993). Ketamine, a drug most similar to tiletamine, is used as the dissociative anesthetic of choice for human infants and has a wide margin of safety has a very short half life, and short duration of action, 20 to 55 minutes in wildlife species studied. No specific studies on excretion in milk of ketamine or tiletamine were found in the veterinary literature but in human neonates, high lipid soluble drugs, of low molecular weight that are non-ionized are estimated to administer 1-2% of the maternal dose to the neonate (Britt and Pasero 1999). The data are limited, but that which are available suggest that excretion of the drugs in Telazol® in milk will not achieve clinically relevant levels in the sucking offspring. Sea lions recovering from injectable anesthetics receive prolonged observation and signs of sedation in the pup would be likewise observed. If any indication of impairment or sedation was noted, the pup could be prevented from entering the water if the risk of the disturbance to other animals on the rookery or haulout was low.

The risk of mortality from the drug combination Telazol® used in the field on Steller sea lions is relatively low 2.3 to 3.9% (but not negligible). No more than 5% capture-related mortality is currently viewed as the maximum acceptable within the wildlife veterinary community and zero mortality when darting free-ranging wildlife has never been realized when more than a handful of animals are captured. The greatest risk for sea lions when using Telazol® is risk of drowning, either by entering the water before the drug takes effect, or the nostrils being submerged in a pool or puddle of water as the animal becomes anesthetized and is not reached in time to move the head before involuntary inhalation. Telazol® administration by darting would only be used in circumstances where no alternative existed (i.e. physical restraint or confinement of the animal prior to hand or pole syringe injection was not possible). Additionally, darting would only be undertaken on an animal that was well positioned to prevent drowning. The animal would need to be high above the waterline, no puddles in the immediate vicinity, positioned with the head directed away from the water, sleeping or at rest with eyes closed. The capture team would be situated to reach the animal immediately upon induction prior to the onset of involuntary respirations. Telazol® is only used in the presence of a wildlife veterinarian or marine mammal biologist with significant experience in darting and the use of Telazol® in free-ranging wildlife. Dr. Kimberlee Beckmen, the wildlife veterinarian responsible for dispensing and supervising the use of capture drugs by Alaska Department of Fish & Game personnel is highly experienced with the administration of Telazol® in wildlife including Steller sea lions, California sea lions, Pacific harbor seals, northern elephants as well as literally hundreds of black bears, wolves, brown bears and various felid species.

Britt R, Pasero C. 1999. Pain control: using analgesics during breast-feeding. Am J Nursing 99(9):20
Hale TW. 199. Anesthetic medications and breastfeeding mothers. J Hum Lact 15:185-194.
Lee JJ and Rubin AP. 1993. Breast feeding and anesthesia. Anesthesia 48:616-625.

4. Additional information should be provided concerning the proposed short- and long-term monitoring of animals to allow reviewers to evaluate whether monitoring will be sufficient to assess the effects of branding/handling on the subject animals.

Response: Effects of handling/branding will be addressed by ADFG as from 2001-2006. Annual survival of branded animals will be estimated from mark-recapture analysis of resighting data collected during annual summer resighting trips by ADFG or NMML in the areas where sea lions are branded. ADFG is committed to resighting surveys throughout Southeast Alaska every summer for the next 10 yrs, given funding is available for this priority work. Health and survival effects of branding will be determined from this data by careful evaluation of brand condition in photographs followed by testing through mark-recapture modeling for survival effects based on healing level of brands. Health effects due to branding/handling will also be assessed by comparing health and condition indices between previously handled and branded animals to those newly captured, during capture studies in which previously branded animals can be opportunistically re-captured. We have monitored short-term effects of

branding/handling on pups marked at rookeries by: weekly resighting surveys to 2-3 months post-branding of branded, branded and tagged, tagged and non-handled pups at Forrester Islands (where enough marked adult females are now available to allow survival of un-handled pups to be determined). We will estimate survival of branded pups to 1-3 weeks post branding at all rookeries in Southeast Alaska using Kendall's robust design, in conjunction with our annual reproductive rate surveys (as done in 2005). Winter surveys will be attempted, when funding allows, to estimate survival of pups to 6 months post-branding (as done in 2003-2004). We will test for variation in survival among branded, tagged, branded and tagged sea lions until 2-3 yrs of age. It is possible this test will be compromised by high rates of tag loss and low resighting rate of tags during our boat based surveys. Analysis of 2001-2002 data from weekly surveys to 3 months post-branding at Lowrie Island, Forrester Islands showed these surveys provide very precise survival estimates with weekly survival estimates ranging from 0.984 – 0.997 and standard errors ranging 0.002-0.005, which were not biased by emigration if additional annual summer data to 3 yrs of age were included in capture histories (Hastings et al. *In Review*). Therefore, we expect high power for tests of branding effects given similar sample size marked per year (200/yr) and continued support for annual brand resighting data collection.

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5. While there may be reasons to use hot branding for permanent identification, the activities in the multiple Stellar [sic] sea lion and northern fur seal applications do not warrant its use. The study design does not require and does not benefit from capture/release/recapture. All biochemical and body morphology do not require multi-year sampling of identified individuals. Any identification needed in one year to prevent resampling of the same animals can be served well by bleach or paint.
Branding should not be used unless it is the only means of identification possible.
Since the protocols do not call for monitoring specific individuals as a key component, hot branding is not acceptable for these permits.

PR1: Please explain why your study cannot be accomplished without hot brands.

Response: The comment is incorrect that our Steller sea lion studies do not warrant hot-branding as a means of permanent identification, and we included a thorough justification of this method on pages 47-65 of our application as "Appendix 1. Justification for and Summary of Hot-iron Branding on Steller Sea Lions Including Branding on Other Pinnipeds." This text was also included in the National Marine Mammal Laboratory application, but presents justification and analysis of this technique applicable to NMML, ADFG, Oregon Department of Fish and Wildlife, Alaska Sea Life Center, and Oregon State University permit applications for Steller sea lion branding.

A repeated observation of individual animals over their lifetime is precisely the reason for using branding. This is the only currently known way to permanently mark pinnipeds with individual marks that can be easily read from a distance, year after year. As stated in Appendix 1 of our application, the hot-iron branding program conducted by

the above groups will provide age-specific survival rates for the eastern and western DPS's (distinct population segment) of Steller sea lions, with the ultimate goal of identifying the age and sex of highest mortality, which may facilitate identification of reasons for decline in abundance. Concomitant to this broader goal will be more detailed determinations of metapopulation age-specific survival rates, age-specific reproductive rates, dispersal from natal rookeries by age and sex, site fidelity, and validation of genetic stock dispersal models. As discussed in Appendix 1 of our application, natural marks, flipper tags, and other marking methods are not acceptable for estimating vital rates of Steller sea lion populations.

We also concur with Dr. Horning's response that "every single scientific peer or panel review conducted over the past 10 years on Steller sea lion research has consistently highlighted the need for multiple studies based on comprehensive, positive, long-lasting individual identification, and specifically branding-based demographic studies. Most reviews and comments received have also underlined the need to continue the ongoing efforts to coordinate multiple research projects. By distributing branding across all projects that manipulate animals in a manner that allows branding, the overall number of animals that need to be captured and manipulated for multiple separate projects can be minimized. Thus, in this case branding LHX animals (and any animal that is manipulated in a manner to permit branding), is in fact directly addressing the REDUCTION components of the three R's (see USDA comments below)" and that "It is important to avoid re-capturing LHX animals for other projects (this consideration actually applies to all projects except for those that specifically use recaptures as part of their experimental design). This is important beyond the first year after release. In particular for underwater captures, brands are the only long-term, reliable positive identification recognizable underwater".

6. In addition, under the AWA, any time a potentially painful or distressful procedure is to be used, alternatives must be researched and final choices must be justified for the study. In this consideration of alternatives, reduction of numbers, refinement of technique, and replacement with other animals must be considered.
If a study exists that requires hot branding (not considered humane), the length of time the branding takes, post procedure treatment and alleviation of pain, and other options must be considered. In the studies under review, there is no justification for using 4 digits, or for branding each digit alone.
At a minimum, 3 digit branding is sufficient to mark more animals that needed for the studies (34X34X34 – over 39,000 combinations, using only 0 and 1, not "o" and "I"). Additionally, devices should be used that load all three irons together so that only one application of the brand is used (20 sec as opposed to 2 minutes).

PR1: Please explain the need for 4 digit brands, i.e., why aren't 3 digit brands used. Please also explain why each digit is applied separately.

Response: The comment reiterates evaluation criteria that must be considered when choosing and justifying the application of potentially painful or distressful procedure on study animals. The ADFG Division of Wildlife Conservation IACUC has reviewed and approved the protocols for animal use for this permit application. Alternatives and

methods for relieving stress and discomfort were addressed as required. Descriptions of the branding method, including the choices of branding digits, possible effects on the animal, and methods employed to reduce stress, pain and suffering were included in the NMML and ADFG permit applications as Appendix 1 entitled "Justification for and Summary of Hot-iron Branding on Steller Sea Lions Including Branding on Other Pinnipeds. This text is also applicable to hot-branding proposed in the Oregon Department of Fish and Wildlife, Alaska Sea Life Center, and Oregon State University permit applications for Steller sea lion studies.

To summarize, many different methods of marking pinnipeds have been developed and tested. Hot branding is currently the only known way to permanently mark pinnipeds with codes that can be easily read from a distance, year after year, throughout the animal's life. In coordination with NMFS National Marine Mammal Laboratory, we have chosen letter designations for our study rookeries (as is done elsewhere throughout the range of Steller sea lions in US and Russian Waters). Each individual pup handled at a rookery is marked with that designator followed by an individual number. Each individual pup handled at a rookery is marked with that designator followed by an individual number (1, 2, 3, …, 43,…..211,……etc.).

The placement of such a large amount of hot iron to the skin of the animal would concentrate far too much heat in the iron cluster, resulting in an inconsistent transmission of heat and greatly increase the risk of over-branding. Even if the reviewer had suggested a new method of hot branding that avoided this increased risk of over branding, it would not be possible to apply more than one mark (number/letter) at a time. Each digit is carefully applied on a specific spot with extreme care and attention to pressure and time of application. Since the animal's body is not flat, a device designed to place all marks at once would remove the ability to monitor each mark and would result in unacceptable variability in the quality of the mark. Finally the application of all digits at one time actually works against one of the methods that we have been trying to use to resolve unreadable brands. We have been purposely "randomly" applying one of the digits off of a straight horizontal line (either high or low) so that it gives us one additional characteristic in the brand photo to compare against future sightings in the event that one of the digits grows/heals to be unreadable. A fixed branding iron would eliminate this additional criteria used to distinguish individuals, thereby reducing resighting ability and ultimately result in a reduced ability to meet the stated objectives.

7. No consideration has been made for post procedure treatment with antibiotics or pain relief has been addressed. All facilities would be required to have an approved protocol from their IACUC that has shown consideration of alternatives and use of methods that would alleviate discomfort, stress, and long-term complications. Topical antibiotic/anesthetic creme should be used post procedure.

PR1: Please explain what considerations have been made for post-procedure analgesia and antibiotics.

Response: The ADFG Division of Wildlife Conservation IACUC has reviewed and approved the protocols for animal use for this permit application. Alternatives and methods for relieving stress and discomfort were addressed as required. All painful

procedures are conducted under general anesthesia. Topical anesthetic creams only penetrate 2mm on mucus membranes, are not recommended for wounds, and have a short duration of effect. Topical anesthetic cream are recommended for use on intact skin for procedures such as catheter placement or blood collection. However, the cream must be covered by an occlusive dressing, takes at least 20 to 30 minutes to reach anesthetic effect, the peak of which is at 2 hours (Mathews 2005). In addition, they are generally water soluble, impair healing and would not have any prolonged analgesic effect once the animal entered the water. Thus, they are not indicated for the types of wounds created in the described procedures. Topical antibiotic ointments are contraindicated for deep puncture wounds (as listed on the label) and thus should not be introduced in to biopsy wounds. Topical antibiotic creams include Nolvasan® (which is water soluble and washes off immediately with water or Silvadene®. Silvadene® is used against bacteria or fungi for human burn patients and could be applied post branding but is normally applied 1 to 2 times daily and kept covered until the wound heals. Thus, applying once, immediately post branding and prior to the post-branding tissue sloughing would probably have a negligible effect but we would not object to its application. We will not use antibiotic ointments or topical anesthetics in the biopsy wounds as this is contraindicated, is contrary to the tenets of wound treatment in veterinary medicine and increases the likelihood of an anaerobic infection and abscessation compared to allowing the wound to heal by second intention.

Injectable analgesics have been considered but the risks and short duration of action outweighed the brief post-procedure benefits. Burtorphanol, a narcotic agonist/antagonist could be administered at a dosage used for domestic carnivores of 0.5 to 0.4 mg/kg subcutaneously. This would likely give some analgesia for 6 to 8 hours. However, it does have the risk of CNS or respiratory depression, either of which we want to avoid in a free-ranging, diving mammal and therefore the risk outweighed the benefits. Buprenorphene is another narcotic and has a longer duration of action, 8 to 12 hours. However it is a controlled narcotic (30 times more potent than morphine) and has a higher risk of CNS and respiratory depression than butorphanol. The non-steroidal anti-inflammatory flunixin meglumine (Banamine®) has been used by marine mammal veterinarians for post-surgical wound pain. It is given at a rate of 1.1 mg/kg IV or IM. The duration of action in pinnipeds is unknown but in carnivores it is given once per day. Dogs are highly sensitive to adverse effects on the gastrointestinal lining (such as gastric ulcers) and is not approved for use in dogs. This is a drug used most often in horses and ruminants. A single dose in a pinniped is probably safe but there isn't any published information on the safety or efficacy in these species. Meloxicam is another NSAID to be considered for juveniles but is excreted at higher concentrations in milk than in plasma in rats so would not be a good choice for the lactating female. There is no recommended dosage for marine mammals so metabolic scaling would need to be employed to estimate a dose. The NSAIDs are not recommended for animals less than 6 weeks of age based on the development of the liver and kidney and should therefore be avoided in rookery aged pups (Mathews 2005). In comparison to the alternatives (i.e. narcotics with respiratory depressive effects), fluninxin meglumine is the best choice we have for juvenile and adults but still carries risks. There isn't a readily apparent safe alternative injectable analgesic for rookery pups indicated for extended analgesia post-branding.

Local anesthesia was considered for analgesia post-biopsy and branding. However, a local infusion could be used and for branding would require such a large area would need to be infused and large volume of lidocaine or buprivacaine that the dose could be toxic. If used it would need to be diluted in saline, buffered with sodium bicarbonate and warmed to body temperature. Additionally, infiltration of lidocaine is extremely painful in the human neonate or pediatric patient (Rodriguez and Jordan 2002) and must be administered very slowly. Animals are likely to have a similar hypersensitivity because of their immature peripheral nerves. An inverted L block could be possible but even for the rookery pup, animals would still need to be anesthestized first (because of the extreme pain of the injection) and held much longer under anesthesia for the prolonged injection time and to wait for minimum 10 minutes for the block to take effect putting the rookery pup at higher risk from prolonged gas anesthesia on a mask without intubation. A spinal block would paralyze the swimming ability and a regional nerve block procedure for pinnipeds is unknown (we don't have the landmarks blocked off). A deep local block for the biopsy is precluded because the presence of the drug would preclude the purpose of the biopsy. The blubber biopsy is for trace contaminants on the ppb level and fatty acid analysis. The presence of the anesthetic and its diluent carrier would negate the accuracy of tissue analyses. In the case of the muscle biopsies, these are to be used for histochemistry and electron microscopy. The local anesthetics and their diluents, are not isotonic, and cause cellular disruptions, especially cell swelling, that would negate the point of the biopsy which is to measure muscle cell fiber diameters. Only a regional nerve block away from the site would prevent these disruptions of the cellular matrix. However, a regional nerve block of the nerves to the pectoralis muscles would paralyze the forelimb which we would not consider doing. To do a nerve block to the area of the dorsal biopsy site, the spinal nerve to that area would need to be infiltrated and would likely paralyze the hind flippers. Again, the risk of these local anesthetic procedures out weigh the benefits of the information obtained from the biopsies. Thus, the IACUC considered the minor post-procedure pain levels to be justified. After conducting and observing hundreds of blubber and muscle biopsies on Steller sea lions, harbor seals, elephant seals and fur seals, I have not been able to detect any indication from the animals of discomfort at the biopsy sites immediately after recovery from anesthesia. They do not look at the site, try to rub it, lick it or have any perceivable difference in limb movement. We do expect that there should be some discomfort based on our own personal experiences but pinnipeds do not seem to show any overt sign of pain post-biopsy. We have observed that some animals post-branding will scratch their neck (not the area of the brand but that is not easily reached) with their rear flipper. This is usually brief and occurs immediately after recovery.

Mathews K A. 2005. Analgesia for the pregnant, lactating and neonatal to pediatric cat and dog. J Vet Emergency Critical Care. 15(4): 273-284.
Rodriguez E and Jordan R. 2002. Contemporary trends in pediatric sedation and analgesia. Pediatric emergency medicine: current concepts and controversies. Emergency Med Clin North Am. 1:199-222.

8. No consideration was given to proper recovery times for anesthesia. While an animal may be awake and mobile within 20 minutes of cessation of isoflurane, it is a recognized occurrence in veterinary medicine that the effects of anesthesia do not dissipate after 20 minutes. In practice, we have seen animals take up to 24-48 hours to recover from anesthesia, especially when the procedure of lengthy (over an hour).

PR1: Please explain what considerations are made for recovery of animals from anesthesia versus the initial return to consciousness.

Response: Although we hold animals over 1 hour, they are never under isoflurane gas for that long. Sea lions are kept on oxygen for 5 to 10 minutes after the isoflurane is discontinued. They are then moved to the recovery area and allowed to breathe ambient air. The anesthetist remains in immediate contact with the animal until it is extubated. After extubation, the anesthetist observes the recovery until the animal is conscious. The animal is allow to remain in the recover area as long as it wants and remains under observation by at least one biologist until it departs. The animal must be fully recovered, very alert and ambulate normally before they leave the recovery area. A physical barrier is used to prevent premature departure until full recover is achieved. Consciousness alone is not criteria for release, they must be alert, responsive and maintaining normal postures. The vast majority of sea lions are fully alert and react normally with a flight response and swim/dive within 20 minutes of the cessation of isoflurane but not all are allowed to depart or try to depart that quickly. If the animal has had a problem, such as a fall during capture or an anesthetic complication, then they are held in a recovery cage or stall until the veterinarian judges the animal is ready for release. Animals have been held over 12 hours as a precaution. In our experience, there has not seemed to be any prolonged recovery from isoflurane and the maximum time of any detectable behavioral sign of sedation when isoflurane has been used alone, is less than 1 hour.

9. While small surface skin biopsies may be acceptable without anesthesia under some conditions, proposals for blubber and muscle biopsies, some up to 2 ½ inches deep, constitute painful and invasive procedures and must be done under anesthesia. If local anesthesia is to be used, dosages should be given, and well as documented protocols for determination of effectiveness, including the waiting period for full effect.

PR1: Please provide details about use of anesthesia for blubber and muscle biopsy, including dosages, how you determined the specific anesthetic/dosage would provide appropriate level of anesthesia, and how long you would wait post-deliver for the anesthetic to take effect. If you do not intend to use anesthesia for these procedures, please explain why.

Response: No blubber or muscle biopsies will be done except under general anesthesia at a surgical plane of analgesia. Refer to Response # 7 for a discussion of post-procedure analgesia.

10. The amount of blood expected to be taken at sampling, while below 10% of blood volume, are significantly high. Given the state of current laboratory methods, it seems that samples can be much smaller, as most tests no longer require 5 ml of serum anymore, more like 0.1 ml. Remember refinement and reduction – this can be applied to sampling as well.

PR1: Please explain why your specific studies require collection of the amount of blood requested in your application.

Response: Reducing blood sampling volumes to a minimum required to achieve analytical and archival needs is among the critical considerations to ensure animal welfare, and we have indeed done so appropriately. Pinnipeds have up to two times the blood volume of terrestrial mammals, and is 90-100 mL/kg for Steller sea lions <30 months old, and about 120 mL/kg for adult females (Richmond et al. 2006). Thus our requested sampling scheme of drawing 1 mL blood/animal kg is a proportion of about 1% of the blood volume of pups-juveniles, and 0.8% of the total blood volume of adult females, an order of magnitude less than a 10% sampling volume. These are extremely conservative sampling rates. By comparison, pediatric human phlebotomy guidelines recommend up to approximately 1.2 mL/kg as a maximum volume to be withdrawn at any one time (Garza and Becan-McBride 1984). Finally, our sampling requirements are determined by volumes required for analyses as directed by the analytical or research laboratories analyzing the samples.

Although the volumes for individual analyses may be very small, there must be consideration for the repetition of analysis within each assay for QA/QC. We are also very committed to trying to archive serum for future research to provide baseline data for retrospective analyses. The following are examples of the volumes of serum or plasma or whole blood required for representative analyses. It should be noted, that given the typical packed cell volume of Steller sea lions, that the volume of whole blood needed to yield this volume of serum or plasma is approximately double.

For example:

Veterinary/clinical chemistry panel – 1.0 ml serum

Deuterium assay for percent body fat - total 9 ml serum (3 ml for pre-sample, 3 ml for post 1 and 3 ml for post 2)

Hydration state – water content of whole blood (0.5 ml whole blood) and of serum (0.5 ml serum)

Metabolite chemistry – β-hydroxybutyrate, blood urea nitrogen, glucose, non-esterified fatty acids, total protein – 2.0 ml plasma total

Blood volume using Evan's blue dye – 8 ml serum (2 ml each for pre, post1, post2 and post3)

Haptoglobin – 0.25 ml serum

Serum iron – 0.5 ml serum

Growth hormone – 0.5 ml serum

Fatty acid signature – 1.5 ml serum

Stable isotope (carbon and nitrogen) – 1.8 ml serum

Chlamydia assay – 0.25 ml serum

Calicivirus assay – 0.5 ml serum

Morbilivirus, distemper, leptospira – 1 ml serum
Parvovirus – 1.0 ml serum
T. gondii - 0.5 ml serum
Brucella – 0.5 ml serum

Garza, D. and K. Becan-McBride. 1984. Phlebotomy handbook. Prentice-Hall, CT. 305 p.

11. As discussed above, all should address the potentially painful procedures and care of the animals.

PR1: Please explain measures that would be taken during and after potentially painful procedures.

Response: All painful procedures are done under general anesthesia in a surgical plane (no perception of pain). Post-operative analgesia options are referred to # 7.

12. Applicants identify that studies have already proven that certain methods are equivalent to the "gold standard" –deuterium(?) measures – for determining body composition. Therefore, it appears to be redundant and unnecessary for more than one method be used, increasing the handling and sedation/anesthesia. Only one method for determination of body composition should be used for each study.
The intent of the studies is to monitor the animals, not compare methodologies.
As an endangered species, these sea lions should be studied, not experimented on.

PR1: Please explain why it is necessary to use multiple methods on the same animal for determining body composition and why comparison of the methods cannot be performed using a non-ESA listed species.

Response: We requested authorization for three procedures that assess body condition. Measuring the dilution of labeled (deuterated) water injected in to an animal allows estimation of the total body lipid stores, yet requires holding times of up to 2.5 hours to allow distribution of the labeled water throughout the total body water pool. In contrast, bioelectrical impedance analysis (BIA) may be an acceptable alternative when conditions preclude the 2.5 hour equilibration period need fro deuterium and passively measures electric resistance of a body, which is related to body water (and thus body lipid) content. The evaluation of this technique must be made on a species-specific basis, and much of the data for that comparison have been collected and are currently being analyzed. A third procedure we utilize is external ultrasonographic determination of blubber thickness. Neither deuterated water nor BIA provide information on blubber thickness, nor does ultrasound provide information on whole body lipid content, so the techniques are not redundant.

Completion of the bioelectrical impedance analysis takes only minutes while the animal is under anesthesia, and has been performed as a backup in the event that the deuterium oxide equilibration is not successful (although admittedly this is an infrequent

occurrence). The deuterium dilution method has been accepted as the most accurate method to use to determine the total body water (and by calculation percent body fat) of individual animals. However there may be instances where it is not possible to hold the animal for the duration of the 2.5 hour deuterium equilibration period and in this case the slightly lower resolution method of bioelectrical impedance analysis could be used to get an estimate of body condition. The comparisons between these methods that have been conducted under previous permits were required because a calibration equation must be developed for each individual species due to slight differences in body shape that impact these empirical relationships.

13. Tooth extraction has not been justified, as there are no aspects of the study that require precise aging. The categories referenced are broad and the experienced and trained researchers should be able to tell approximate age without having to pull a tooth and the dangers the procedure entails (anesthesia, malocclusion, dental abscesses, pain).

PR1: Please explain why your study requires the precision age determination associated with tooth extraction rather than relying on other methods for grossly estimating age.

Response: It is incorrect to state that no aspects of the study require precise aging. One of the fundamental needs to understanding the reasons for the decline of Steller sea lions is the ability to recognize whether a threat is specific to a particular cohort or age class. There are currently only two methods of reliable aging in all age classes of Steller sea lions: 1. aging by counting the incremental growth layers on a longitudinal section or 2. by permanently marking the animal at a known age and then resighting the animal throughout it's life. In addition, one of the most useful measures of body condition is the mass or size of a sea lion relative to its age. Linking data collected on foraging behavior, health and condition to a known-age has and continues to be a critical component of our studies. Past evidence for decreased body condition was based on size-at-age, and long-term and spatial comparisons depend upon accurate estimates of age. As juveniles were considered to be an age-group most vulnerable to shifts in prey abundance, the development of their physiological and behavioral capabilities has been a central focus. The ages of pups and juveniles captured under the current permit are estimated based on a combination of teeth eruption pattern, general animal size, and season of capture. This technique becomes much less precise after a sea lion is older than one year. Based on King et al. (2007) it appears that tooth extraction may no longer be necessary for animals <2 years old to determine age and the necessity for this procedure has been reduced to animals greater than 2 years of age that have not been previously branded.

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14. The section on determination of sample sizes (page 6) states that they were chosen based on 20 animals "per 3 month age category/bin." This does not appear to be reflected in the summary charts, nor is it adequately explained in the text.
The applicants also explain that 300 female pups was a sample size adequate for providing data for the study. They outline the difficulty of determining sex prior to

capture and then state that 300 pups total (likely to include substantially fewer than 300 females) will be captured. This number is stated to provide sufficient statistical precision while minimizing wide scale disturbance to the population. We commend the applicants for their concern with increasing disturbance but believe there should be a discussion of why the capture of an unknown (potentially small) number of females is a sufficient substitute for a sample size of 300 as dictated by the branding workshop that they cite. If there is no means of assuring that a smaller sample size will be statistically significant (and no evidence is provided that it will be) then, to avoid risk to animals for no purpose, perhaps none should be branded until this can be assured.

Response: ADFG would like to mark 200 pups per rookery, given at least 1-3 yrs are skipped between years of marking per site. This figure is based on actual data collected 2001-2006 rather than simulations from an earlier brand workshop based on best guesses, and therefore provides the best guidelines for number of marks required for given levels of precision. Results from mark-recapture analysis of 2001-2006 data show data cannot be pooled among rookeries or sexes and that survival is sex- and rookery-dependent in the eastern and western stocks (NMML unpublished analyses, Hastings et al. 2006). From 2001-2005 an average of 190 pups per rookery were marked (ranging from 94 to 291/rookery/year) at 3 of 4 Southeast rookeries, which produced mark-recapture survival estimates ranging from 0.513 to 0.971, with standard errors ranging from 0.013 to 0.040 (ADFG unpublished data). Standard errors for first-year survival estimates of females averaged 0.035 at sample sizes of 150 versus 0.020 for sample sizes of 550 (data pooled over years; ADFG unpublished data). Data from 2001-2006 were not sufficient to detect annual variation in survival probabilities for these ages; and data from one rookery (Graves Rock) had insufficient sample size ($n = 93$) to estimate survival probabilities. Therefore given resight effort continues at a similar level to that of 2001-2006, a sample size of 200 per rookery per year would be necessary to ensure standard errors of < 0.035 and potentially estimate year-specific survival. Year-specific estimates are needed to test for population level responses of Steller sea lions to changes in environmental conditions and test hypotheses concerning what factors in the ecosystem are driving population dynamics of this species.

15. On page 7, the applicants state they will coordinate with two other permittees engaged in capture activities. But there are others who have requested captures including Horning and Trites. There should be coordination with these permittees as well.

Response: In the past NMML and the ASLC have been the only other groups actively undertaking field captures of Steller sea lions, with other researchers collaborating on those projects. As the number of permittees requesting to do field captures (and any research) on Steller sea lions increases, we will of course include these researchers in our coordination activities. Dr. Rea is a collaborator on projects (and a co-PI on the UBC permit application) with these researchers and all were included in our annual research coordination meeting (reported by NMML) held in January 2007. Dr. Rea also sits on the Scientific Review panel of the NPUMMRC with Drs. Horning and Trites and on the

Scientific Advisory Committee of the ASLC, which also provides an additional avenue for communication and coordination.

16. With regard to capture and restraint, the applicants indicate that pups are "restrained by hand or by gas anesthesia if hot branded." Juveniles are restrained "physically or chemically (valium or gas anesthesia);" and adults are said to be "restrained physically, chemically, with gas anesthesia or a combination of the above based on the judgment of the attending veterinarian." (page 14) Yet page 16 indicates that adults are placed in a "squeeze cage that restricts movement without the need for immobilizing drugs." Page 23 states that all animals over 3 years of age will be branded under anesthesia. This varied verbiage and the summary charts (which lump both physical and anesthetic restraint methods together), make it impossible to determine whether animals are receiving proper sedation and/or analgesia for branding and other potentially stressful and painful procedures. This should be clarified and all animals should be treated humanely.
The section on mitigation (page 37) states that sedated animals will be "observed closely after gas anesthesia to ensure full recovery." The time period for observation was not indicated.

Response: The difference in wording in these various sections is due to the fact that in most instances animals are first physically restrained, and then chemically restrained. However, if it is decided after physical restraint that no intrusive procedure is warranted on a particular animal (such as a young pups less than 20 kg that will not be branded) then no chemical restraint will be employed. All painful procedures (including branding) are done under general anesthesia in a surgical plane (no perception of pain) as outlined in our approved IACUC protocols. Post-operative analgesia options are referred to Response # 7. Recovery times and observation protocols are discussed above under Response #8

17. Page 21 discusses the use of fecal loops and states that they will only be used on anesthetized animals, yet it proposes to use this procedure on virtually all captured animals over 2 months of age (Table 1). As noted above, it is not clear that all captured animals will receive anesthesia. Will the applicant avoid this procedure for all nonanesthetized animals and, if so, how will that affect sample size requirements? Or will all animals in fact be anesthetized, despite the conflicting verbiage in the sections under restraint and hot-branding?

Response: Fecal loops will only be used on anesthetized animals, and since there are very few instances in which we have not anesthetized animals over 2 months of age once captured, this technique has the potential to be used on most of the animals requested for capture. The small number of exceptions will not impact the study design, since the occurrence of parasites in these populations will be impacted to a much lesser extent than other indices we are concerned about monitoring seasonally (such as percent body fat content).

18. We note that this applicant proposed both flipper tagging and branding. Applicant Horning argued that these temporary marks were duplicative and unnecessary for branded animals. Can the applicant discuss why they feel that procedure this is necessary?

Response: We do not intend to apply both brands and flipper tags to the same individuals. Flipper tags will only be used in the case where an animal has been judged to be unsuitable to brand. Two examples: pups less than 20 kg will not be branded, and if an older animal is showing poor response to anesthesia it will be revived immediately and released with flipper tags instead of a brand. This is described more fully under section *h. Flipper tag or other mark* on page 20 of our application. However, we may need to use a temporary mark (such as a livestock marker mentioned in our application) to denote a previously branded animal than has been handled during the current capture trip that would allow us to easily avoid an underwater recapture of that individual during that research trip.

19. With regard to text on pages 27 proposing darting animals with Telazol, we reiterate our comment made under the NMML permit regarding deaths of darted animals. Though few deaths were observed, a very large number of animals either moved to inaccessible areas or went into the water, making it impossible to learn their fates. The discussion provided is no assurance that this is not a risk prone method for delivering sedation, and provides evidence that it is in fact risky. It should not be allowed. See 50 C.F.R. § 216.34(a)(1) (the applicant must demonstrate the that activity is "humane and does not present any unnecessary risks to the health" of the animal).

Response: Please see discussion under Response #3.

20. The discussion of mortality beginning on page 33 omits discussion of the paucity of postprocedure monitoring. Given the Oregon study, cited by this applicant, that found significant differences in survival of branded pups, the discussion of previously noted deaths is not sufficient assurance that additional deaths did not occur in the absence of subsequent monitoring. We reiterate our comments on the inadequate accounting of incidental mortality that we provided on the NMML permit, as the verbiage here is virtually identical.

Response: Please see discussion under Response #4 that outlines our significant efforts at post-procedure monitoring.

21. We appreciate the appendix that discusses branding and resighting and are pleased to see that there has apparently been an increase in resighting activities such that vital rate estimates are being generated with greater precision that previously (see DEIS discussion on the lack of effort since 1975 and the inability to determine survivorship for adults). The discussion of "pain and suffering" omits information on the nature and

degree of pain that is contained in the DEIS and in information provided by applicant Trites who proposes to study manifestations and mediation of pain and stress. The discussion also omits mention of Scordino's results from an Oregon study that found adverse effects from branding of pups that led to increased mortality. There is also no acknowledgement of the general lack of post-procedure monitoring that is admitted in the DEIS, nor its effect on understanding of levels of indirect mortality. There is no accounting for mixed reviews of the effects of branding of elephant seals on Macquarie Island and the banning of this practice by the governments.

Response: The ADFG Division of Wildlife Conservation IACUC has reviewed and approved the protocols for animal use for this permit application. Alternatives and methods for relieving stress and discomfort were addressed as required. All painful procedures are conducted under general anesthesia in a surgical plane (no perception of pain). It should also be noted that the DEIS was not yet published at the time of our permit application in December 2006. The Oregon study was conducted at a site in the Eastern stock and clearly stated in the conclusions that all estimates were based on "apparent survival" and confounded by emigration. Studies on Ugamak Island and Lowrie Island have not indicated similar effects. Additional in-depth discussion of branding and potential alternatives is available in Appendix 1 entitled "Justification for and Summary of Hot-iron Branding on Steller Sea Lions Including Branding on Other Pinnipeds. Please also see discussion under Response #4 that outlines our significant efforts at post-procedure monitoring.

Comments received on File No. 881-1890 (Alaska SeaLife Center, Steller sea lion research)

Marine Mammal Commission (MMC)

1. In discussing the potential effects of Telazol on nursing pups, page 54 of the application states that "[T]elazol has been shown to cross the placental barrier and therefore use of Telazol for Cesarean section in dogs and cats is contraindicated (Telazol drug information sheet; CI 5129-1; Fort Dodge Animal Health, Fort Dodge, IA). However, the application goes on to state that "[T]elazol, however, causes less respiration depressing in the fetus than other commonly used injectable anesthetics, and therefore it is commonly used for Cesarean sections in monkeys and cats (M. LaRosh, D.V.M.; Fort Dodge Animal Health Veterinarian, Pers. Comm.)." The applicant should address the apparent discrepancies in these two statements.

Response: The makers of Telazol have not conducted controlled trials to determine the effects of its use on fetuses. Therefore, because it is possible for Telazol to cross the placental barrier their product information sheet cautions against its use in pregnant dogs and cats. However, the Fort Dodge Animal Health Veterinarian stated that in clinical practice, it is common to use Telazol for Cesarean sections in monkeys and cats and that the small amount of respiratory depression observed in the fetus does not cause complications. We are only proposing to use Telazol on adult female Steller sea lions on the rookery, when they will either not have a fetus (between parturition and copulation) or will have an unimplanted blastocyst that hasn't developed a respiratory system. Therefore, respiratory depression in the fetus is not a possibility in our study.

2. Additional justification should be provided for the proposed at-sea foraging study that involves buoyancy/drag experiments on females with dependent pups.

Response: We aim to determine how variations in the prey field can affect Steller sea lions. In order to do this, we will rely on two approaches: examination of foraging behavior in areas of varying prey field and experimental manipulations. We cannot control the prey field that is available to Steller sea lions, but it is clear that experimental manipulations are an extremely powerful way to obtain answers to these sorts of questions. An alternative to experimentally reducing prey density or changing the prey field composition to one with lower food quality is to experimentally increase the cost of foraging by increasing the hydrodynamic drag. This is a feasible manipulation that can be done for a very brief period of time, but this can provide valuable information on the ability of Steller sea lions to compensate for additional costs of foraging, such as might occur if prey density or quality is reduced. Neglecting to study lactating Steller sea lions would handicap our efforts to provide knowledge that can be used to aid the recovery of Steller sea lions. An experimental manipulation that can be properly controlled and replicated is the only way that we will be able to meet many goals of the Steller sea lion Recovery Plan. This was emphasized in many of the Peer Reviews of the Steller sea lion research plans, as well as in Bowen et al. (2001; Review of the November 2000 Biological Opinion and Incidental Take Statement with Respect to the Western Stock of the Steller Sea Lion, with Comments on the Draft August 2001 Biological Opinion). The Bowen report recommended experimental manipulations of fishing effort in Steller sea lion habitat in order to examine the effects of such stress on sea lions, but NMFS has determined that such experiments are impractical. A reasonable alternative, however, is to experimentally stress individual sea lions by changing the cost of

foraging, which we can achieve by altering buoyancy or hydrodynamic drag. Such an increase in foraging costs can be compared with the increased cost that might be incurred by sea lions if prey density was reduced due to fishing effort or climate change. It has been suggested that some pinnipeds are routinely operating well within their physiological limits and may easily be able to cope with environmental stress by working a little harder or spending more time at sea searching for fish (Costa et al. 2001). However, some species of otariids seem to be working near their physiological limits, which may constrain their ability to respond to environmental change.

3. The application states that "buoyancy/drag blocks will be left on [lactating females] for no more than four weeks" and that "the slight changes in drag and/or buoyancy will not significantly affect predator escape responses" or have "any significant adverse, long-term effects on female body condition, ability to provision young, or survival." The applicant should explain what it considers to be a "significant" adverse effect and explain why it believes that no such effects would occur.

Response: A significant adverse effect would be mortality of the instrumented subject or the failure of the lactating female to successfully wean its pup. In a study that fitted lactating Antarctic fur seal females with similarly proportioned drag devices, characteristics of individual dives were affected and foraging trip length increased by 10% but maternal mass and pup growth were not different between control and experimental groups (Boyd et al. 1997).

4. Information also should be provided concerning potential effects of buoyancy experiments on juvenile Steller sea lions.

Response: We expect to see similar results as observed in studies where buoyancy of penguins and elephant seals were altered. In those studies the characteristics of individual dives were affected, such as ascent and descent rates, but the individuals were able to compensate for the changes in buoyancy and foraging success was not altered. Therefore, although we expect to gain insight into how Steller sea lions cope with changes in buoyancy (due e.g., to changes in body condition or instrumentation) or work load (increased costs due to changes in fish distribution or instrument attachment), we do not anticipate that these changes will adversely impact individuals in a significant way.

5. In addition, the application states that buoyancy/drag blocks will be left on female and juvenile Steller sea lions for no more than four weeks, at which time they will be removed with remote-release devices. The applicant should provide information concerning what would be done to remove the blocks if the remote-release device fails.

Response: The blocks will be attached with a back-up safety release consisting of a cable that corrodes in approximately 2 months.

6. In describing the floating platform method of capture, the application states that sea lions hauled out on the platform are free to come and go until they are captured and transferred into a holding cage on a 30-foot barge. They are then moved one at a time from the holding cage into a stainless steel squeeze cage. The application states that "[s]ea lions that are released from the cage without any sampling or other restraint are considered to be incidentally disturbed." An animal being held in a cage has effectively been captured

and should be considered as such. The applicant also should provide information on the maximum duration animals would be maintained in the holding cage before they are sampled or released.

Response: The Office of Protected Resources had accepted this definition of "capture"in our previous permit amendment. However, we agree that once the door on the floating trap has been closed, the sea lions in the trap are effectively captured even if we do not handle them. If the permit office agrees, we suggest that a new category of take be added to our permit: "Capture by floating trap and release without handling". This category would include only those sea lions that were in the floating trap when it's door was closed, but which were not put into a squeeze cage or handled in any other way before the door was re-opened so that they could leave the cage. We anticipate that for every sea lion captured and handled, another 4 will have to be released unhandled from the trap. If all of our captures were performed using the floating trap, then we would have to multiply our total number of captures for Task 1 by 4. This would result in the following additional takes, for the category of "Capture by floating trap and release without handling".

Pups 2 mo. to 1 yr: 480

Sea lions>1yr. to 4 years: 480

Adult females: 1200

Animals (juveniles) captured on the floating platform for the Transient Juvenile project (Task 2) would be held in the holding cage only long enough to transfer the transport enclosure to the skiff for transportation to the ASLC quarantine facility (<1hour).

7. The application states that "[w]e plan to recapture adult females and juveniles twice during a year and to recapture pups as many as four times annually (no more than once per week)." A discussion of the potential for adverse impacts on pups or disruption of the mother/pup bond as a result of such frequent activities is not, but should be, provided.

Response: Previous studies have been conducted in which Steller sea lion pups were captured at least 3 times within a month and there was no evidence that this led to a severing of the mother/pup bond or an adverse impact on the pup (Brandon et al., 2005).

8. Additional information needs to be provided regarding the potential for adverse impacts to the subject animals and the potential for biasing the research results by subjecting the pups to the repeated stress of capture and handling.

Response: As mentioned above, capturing Steller sea lion pups at least 3 times within a month did not lead to a severing of the mother/pup bond or an adverse impact on the pup (Brandon et al., 2005).

9. As a related matter, whereas the text states that pups would be recaptured up to four times annually, Table 1 accompanying the application indicates that pups would be taken up to five times annually. This apparent discrepancy should be resolved.

Response: This is not a discrepancy. The take table lists the total number of takes per individual, so an initial capture plus recapturing up to four times equals five total takes.

10. Further, Table 1 and the other tables should be re-titled to reflect that the take numbers listed are annual numbers.

Response: The column heading for the takes is titled: Expected Annual Take import/export.

11. The application states that "[a]ll procedures included will only be performed under valid ASLC IACUC approvals. Copies of these approvals will be provided prior to any sampling event." The applicant should provide documentation of Institutional Animal Care and Use Committee approval prior to issuance of a permit.

Response: Copies of relevant IACUC approvals will be provided to PR1 for their records.

12. In discussing activities proposed under the Transient Juvenile Steller Sea Lion Project, the application states that "[w]henever possible, most procedures, including but not limited to blood and tissue collection, whisker extraction, hot-branding, attachment of scientific instruments and x-ray, will occur while the animal is under general anesthesia in order to reduce potential stress to the animal…." The applicant should explain and justify under what conditions anesthesia would not be used.

Response: To clarify this point, only blood collection and attachment of scientific instruments may occur without gas anesthesia, and only under exceptional circumstances. In some cases, it is more efficient and less stressful to manually restrain an individual if there is only a short period of contact required. For instance, a skilled individual may be able to collect a small blood sample for diagnostic purposes without the need for anesthesia. In the event that the Attending Veterinarian and Chief Scientist agree that an animal may be safely restrained (e.g., in a squeeze cage) for a short period of time (<10min) to allow for either or both of these procedures without adding the risk of gas anesthesia, this will be deemed the preferred option. Although gas anesthesia in general is considered a safe procedure that is utilized to reduce the stress and increase safety for most handling and collection events, there is inherent risk to the procedure that in a few selected cases is not necessary.

13. The application states that deuterium oxide would be administered to juvenile sea lions up to four times to monitor body condition during temporary captivity. The application states that "[a]nimals may be maintained under anesthesia for the duration of the equilibration period [approximately 120 and 135 minutes] or manually restrained via squeeze cage for post-D_2O blood samples." The applicant should describe (1) what criteria would be used in deciding whether or not to anesthetize animals for this activity, (2) over what time intervals deuterium oxide studies would be conducted, and (3) any potential consequences of repeatedly anesthetizing animals for this purpose.

Response: (1) As stated in the previous response, there are specific circumstances where a skilled individual may be able to safely collect the required blood sample with the use of a squeeze cage only. These events would be limited to procedures that required 10min or less of handling time. In the case of deuterium oxide, this would be limited to the collection of pre-injection blood (5-10ml), or post-injection blood samples (5-10ml each). Injection would occur only under gas anesthesia. Typically, deuterium oxide is administered in conjunction with other sampling events to minimize handling such that these cases would be rare.
(2) Deuterium oxide dilution methodology is used for the non-lethal determination of body condition at a minimum of two points per individual. Body condition (ie., fat content) is assessed at entry into the Transient Research Project to give a more accurate picture of the overall health of the animal at capture, and again prior to exit in order to ensure adequate fat stores prior to release. There are some experimental protocols that require additional administrations of deuterium for intermediate body fat estimates, such as diet studies,

restricted intake, metabolic studies and fasting studies. These events will be combined with other procedures to reduce the overall handling. The minimum interval would be one week, which corresponds to the maximum of weekly health assessments.
(3) The risk inherent with anesthesia is present at induction and does not necessarily increase with duration of anesthesia. Therefore, combining deuterium events with weekly health assessments, as is done will all procedures to the maximum extent possible, does not increase the consequences of anesthesia. Repeated gas anesthesia, even on a daily basis, is a routine veterinary procedure used on animals of all species to reduce stress and pain as necessary (eg. Radiation treatments, painful re-bandaging, dealing with dangerous animals) and has no cumulative effect due to anesthetic drug use (B. Heath, pers.com). Complications which might arise due to abnormal blood circulation, respiratory effort or body temperature are prevented by routine anesthetic support measures such thermal support and periodic positive pressure ventilation and repositioning.

14. The application states that during the Transient Juvenile Steller Sea Lion Project, up to 12 juvenile sea lions will undergo up to two ten-day fasting events (partial or full food restriction) spaced a minimum of two weeks apart. The application states that metabolic chamber readings of basal metabolic rate and blood samples will be collected up to four times during each fasting period, and that animals would be manually restrained in a squeeze cage or anesthetized for the procedure. The applicant should describe (1) what criteria would be used in deciding whether or not to anesthetize animals for this activity, and (2) any potential consequences of repeatedly anesthetizing animals for this purpose.

Response: (1) As stated in the above two responses, in the event that the required blood sample can be obtained with a minimum amount of handling (<10min) and without the small potential of added risk of anesthesia, we will chose manual restraint as the lesser risk. If procedures are likely to take longer, or the animals are of a size or temperament to make restraint dangerous for the animals or the staff, then anesthesia will be chosen as the lesser risk. Metabolic chamber readings occur while the animal is alert and therefore do not require anesthesia.
(2) As stated in the previous two responses, the inherent risk, although small, with gas anesthesia is highest during the induction period and no cumulative effects are anticipated. To the maximum extent possible, we will combine research sampling events with weekly health assessments to reduce the total handling and anesthesia time per individual. At most, the individual would encounter 2 additional anesthesia events over the 12 weekly health assessments. It should also be noted that whenever possible, we operate on a bi-weekly health assessment to further reduce the amount of handling and anesthesia. In the past, we have been able to successfully complete research requirements on this reduced schedule for approximately half of the animals through the program. Every effort is made to reduce both the number of samples taken and the amount of handling for every individual while maintaining the required research priorities.

15. Page 41 of the application states that "[i]mplantation of dual life history transmitters will be performed with a minimum of three people: a surgeon, an anesthetist and a non-sterile surgical assistant." However, page 42 states that "[o]nly qualified veterinarians or other personnel with sufficient experience (e.g., Wildlife Biologists with >5 years of surgical experience) in the technique will be allowed to perform this procedure [surgical implants

of dual life history transmitters]." Assuming that a surgeon would be a veterinarian, the applicant should address this discrepancy. The applicant also should provide justification of why an experienced marine mammal veterinary surgeon, or a veterinarian with extensive surgical experience working under the supervision of an experienced marine mammal veterinarian, would not carry out this surgical procedure.

Response: Only experienced veterinarians will perform this procedure. Experienced Wildlife Biologists (>5yrs of surgical experience) may, however, be part of the supporting personnel to assist the Surgeon/Attending veterinarian.

USDA Animal and Plant Health Inspection Service (APHIS)

16. While there may be reasons to use hot branding for permanent identification, the activities in the multiple Stellar [sic] sea lion and northern fur seal applications do not warrant its use. The study design does not require and does not benefit from capture/release/recapture. All biochemical and body morphology do not require multi-year sampling of identified individuals. Any identification needed in one year to prevent resampling of the same animals can be served well by bleach or paint.

    Branding should not be used unless it is the only means of identification possible. Since the protocols do not call for monitoring specific individuals as a key component, hot branding is not acceptable for these permits.

PR1: Please explain why your study cannot be accomplished without hot brands.

Response: The hot-branding protocol is a highly-collaborative effort adhered to by multiple agencies, including ASLC, ADF&G, ASLC and ODFW. As such, our response below, as well as with the subsequent concern on the number of digits utilized, is presented as a joint effort with agreement from all agencies working together.

A repeated observation of individual animals over their lifetime is precisely the reason for using branding. This is the only currently known way to permanently mark pinnipeds with individual marks that can be easily read from a distance, year after year. The hot-iron branding program conducted by the above groups will provide age-specific survival rates for the eastern and western DPS's (distinct population segment) of Steller sea lions, with the ultimate goal of identifying the age and sex of highest mortality, which may facilitate identification of reasons for decline in abundance. Concomitant to this broader goal will be more detailed determinations of meta-population age-specific survival rates, age-specific reproductive rates, dispersal from natal rookeries by age and sex, site fidelity, and validation of genetic stock dispersal models. As discussed in Appendix 1 of NMML's application, natural marks, flipper tags, and other marking methods are not acceptable for estimating vital rates of Steller sea lion populations.

Specific to ASLC's Task 2, the Transient Project, hot-branding is a crucial means for post-release monitoring for highly-studied individuals. These animals in particular represent juveniles with well-documented histories, but also have been subject to multiple research events. The only way to completely ensure that these animals are correctly identified if recaptured or resighted by collaborating agencies is to use a permanent brand, which is to the advantage of the individual (such that it is not subject to multiple invasive procedures over their lifetime) and to the researcher (to be able to utilize previously gathered data to the best advantage).

In addition, under the AWA, any time a potentially painful or distressful procedure is to be used, alternatives must be researched and final choices must be justified for the study. In this consideration of alternatives, reduction of numbers, refinement of technique, and replacement with other animals must be considered. If a study exists that requires hot branding (not considered humane), the length of time the branding takes, post procedure treatment and alleviation of pain, and other options must be considered. In the studies under review, there is no justification for using 4 digits, or for branding each digit alone.

At a minimum, 3 digit branding is sufficient to mark more animals that needed for the studies (34X34X34 – over 39,000 combinations, using only 0 and 1, not "o" and "I"). Additionally, devices should be used that load all three irons together so that only one application of the brand is used (20 sec as opposed to 2 minutes).

PR1: Please explain the need for 4 digit brands, i.e., why aren't 3 digit brands used. Please also explain why each digit is applied separately.
Response: The comment reiterates evaluation criteria that must be considered when choosing and justifying the application of potentially painful or distressful procedure on study animals. Descriptions of the branding method, including the choices of branding digits, possible effects on the animal, and methods employed to reduce stress, pain and suffering were included in the NMML and ADFG permit application appendices entitled "Justification for and Summary of Hot-iron Branding on Steller Sea Lions Including Branding on Other Pinnipeds. This text is also applicable to hot-branding proposed in the Oregon Department of Fish and Wildlife, Alaska Sea Life Center, and Oregon State University permit applications for Steller sea lion studies.

To summarize, many different methods of marking pinnipeds have been developed and tested. Hot branding is currently the only known way to permanently mark pinnipeds with codes that can be easily read from a distance, year after year, throughout the animal's life. In coordination with NMFS National Marine Mammal Laboratory, we have chosen letter designations for our study rookeries (as is done elsewhere throughout the range of Steller sea lions in US and Russian Waters). Each individual pup handled at a rookery is marked with that designator followed by an individual number. Each individual pup handled at a rookery is marked with that designator followed by an individual number (1, 2, 3, …, 43,…..211,……etc.).

The placement of such a large amount of hot iron to the skin of the animal would concentrate far too much heat in the iron cluster, resulting in an inconsistent transmission of heat and greatly increase the risk of over-branding. Even if the reviewer had suggested a new method of hot branding that avoided this increased risk of over branding, it would not be possible to apply more than one mark (number/letter) at a time. Each digit is carefully applied on a specific spot with extreme care and attention to pressure and time of application. Since the animal's body is not flat, a device designed to place all marks at once would remove the ability to monitor each mark and would result in unacceptable variability in the quality of the mark. Finally the application of all digits at one time actually works against one of the methods that we have been trying to use to resolve unreadable brands. We have been

purposely "randomly" applying one of the digits off of a straight horizontal line (either high or low) so that it gives us one additional characteristic in the brand photo to compare against future sightings in the event that one of the digits grows/heals to be unreadable. A fixed branding iron would eliminate this additional criteria used to distinguish individuals, thereby reducing resighting ability and ultimately result in a reduced ability to meet the stated objectives.

17. No consideration has been made for post procedure treatment with antibiotics or pain relief has been addressed. All facilities would be required to have an approved protocol from their IACUC that has shown consideration of alternatives and use of methods that would alleviate discomfort, stress, and long-term complications. Topical antibiotic/anesthetic cream should be used post procedure.

PR1: Please explain what considerations have been made for post-procedure analgesia and antibiotics.

Response: The internal ASLC IACUC has been tasked with ensuring that all considerations have been taken into account when dealing with pain and discomfort. All procedures listed in the application for Task 2, the Transient Project, are currently under active AUP/IACUC approval such that these considerations have been undertaken. Copies of the relevant approvals will be provided to PR1. Application of topical preparations would add no benefit to the healing process and would require repeated stressful handling of the animals as it would be rapidly removed from the skin when the animals self groomed or entered the water. We would also direct the commenter to NMML's detailed response to this concern if they require additional information.

18. No consideration was given to proper recovery times for anesthesia. While an animal may be awake and mobile within 20 minutes of cessation of isoflurane, it is a recognized occurrence in veterinary medicine that the effects of anesthesia do not dissipate after 20 minutes. In practice, we have seen animals take up to 24-48 hours to recover from anesthesia, especially when the procedure of lengthy (over an hour).

PR1: Please explain what considerations are made for recovery of animals from anesthesia versus the initial return to consciousness.

Response: Animals are closely monitored throughout the anesthetic recovery process from the initial return to consciousness through the ability to move and react normally. In 10 years of experience with over 400 isoflurane procedures on captive Steller sea lions (most of which lasted greater than 2 hours) we have consistently observed recovery to full function in 30 to 60 minutes as long as no other sedative medications are used (P. Tuomi, DVM, pers com). Due to the logistical advantages of the quarantine facility, animals are allowed to recover in a dry location. Access to water is not granted until the Chief Scientist and Attending Veterinarian deem it safe based on the stable movement and normal behaviors (i.e., grooming, response to visual stimulation, interest in food, etc.) of the individual. During field capture situations, animals are allowed to recovery in the safety of the capture or transport box. When the animal is deemed safe to release, the door is opened such that the animal can leave of its own will when ready.

19. While small surface skin biopsies may be acceptable without anesthesia under some conditions, proposals for blubber and muscle biopsies, some up to 2 ½ inches deep,

constitute painful and invasive procedures and must be done under anesthesia. If local anesthesia is to be used, dosages should be given, and well as documented protocols for determination of effectiveness, including the waiting period for full effect.

PR1: Please provide details about use of anesthesia for blubber and muscle biopsy, including dosages, how you determined the specific anesthetic/dosage would provide appropriate level of anesthesia, and how long you would wait post-deliver for the anesthetic to take effect. If you do not intend to use anesthesia for these procedures, please explain why.

Response: For research under both Task 1 and Task 2, all muscle and blubber biopsies will only take place while the animal is under general gas anesthesia, which provides complete pain relief for these procedures.

20. The amount of blood expected to be taken at sampling, while below 10% of blood volume, are significantly high. Given the state of current laboratory methods, it seems that samples can be much smaller, as most tests no longer require 5 ml of serum anymore, more like 0.1 ml. Remember refinement and reduction – this can be applied to sampling as well.

PR1: Please explain why your specific studies require collection of the amount of blood requested in your application.

Response: Sampling regimes for the Transient Project, Task 2, are listed separately from Task 1 due to the unique nature and repeated sampling protocols during the temporary captivity period. Blood collection for Task 2, the Transient Project, is limited to the lesser of 1% total body mass (e.g., 1cc/kg body mass) or cumulative 5% total body mass per month as is well below accepted veterinary standards (e.g., Murray 2000), as noted in our application (p. 43). Blood collection techniques and volumes are quite standard among collaborating agencies. As NMML's accurate response to this concern notes, 'This comment suggests unfamiliarity with pinniped blood volumes, our requested sampling volumes, and with volumes required for laboratory analyses. Reducing blood sampling volumes to a minimum required to achieve analytical and archival needs is among the critical considerations to ensure animal welfare, and we have indeed done so appropriately. Pinnipeds have up to two times the blood volume of terrestrial mammals, and is 90-100 mL/kg for Steller sea lions <30 months old, and about 120 mL/kg for adult females (Richmond et al. 2006). Thus our requested sampling scheme of drawing 1 mL blood/animal kg is a proportion of about 1% of the blood volume of pups-juveniles, and 0.8% of the total blood volume of adult females, an order of magnitude less than a 10% sampling volume. These are extremely conservative sampling rates. By comparison, pediatric human phlebotomy guidelines recommend up to approximately 1.2 mL/kg as a maximum volume to be withdrawn at any one time (Garza and Becan-McBride 1984). Finally, our sampling requirements are determined by volumes required for analyses as directed by the analytical or research laboratories analyzing the samples. While some analyses may require small amounts of plasma or serum, non-clinical analyses are typically run in duplicate or triplicate, and because pinnipeds have much higher hematorcrits than terrestrial mammals the expansion to the whole blood volume that must be collected to result in sufficient plasma or serum is larger than expected by those familiar with routine laboratory animal sampling. Thus, our sampling requirements range from 1 mL whole blood for analyses of leptin for haptoglobin levels, through up to 16 mL total withdrawal for deuterated water determination of body composition.'

21. As discussed above, all should address the potentially painful procedures and care of the animals.

PR1: Please explain measures that would be taken during and after potentially painful procedures.

Response: As stated above in the previous question, all procedures are subject to rigorous internal review by the ASLC IACUC such that all protocols that may involve pain or distress have been scrutinized. All procedures listed in the application for Task 2, the Transient Project, are currently under active AUP/IACUC approval.

22. Applicants identify that studies have already proven that certain methods are equivalent to the "gold standard" –deuterium(?) measures – for determining body composition. Therefore, it appears to be redundant and unnecessary for more than one method be used, increasing the handling and sedation/anesthesia. Only one method for determination of body composition should be used for each study. The intent of the studies is to monitor the animals, not compare methodologies. As an endangered species, these sea lions should be studied, not experimented on.

PR1: Please explain why it is necessary to use multiple methods on the same animal for determining body composition and why comparison of the methods cannot be performed using a non-ESA listed species.

Response: For Task 2, the Transient Project, we perform multiple assessments of body condition that provide different information. We utilize deuterium oxide for total body fat and ultrasound for localized non-invasive measurements of blubber depth. In addition, we are validating the use of thermal imaging in conjunction with these two methods to assess the capabilities of the technology to measure body fat/condition remotely in a rookery/haulout environment without the need for animal handling.

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23. The application proposes a novel method to test how decreased prey availability may affect Steller sea lions by altering the hydrodynamic drag and buoyancy of free-ranging juveniles and adult females. If, as the application states, these simulated changes in work load and body condition are short term and unlikely to cause no significant adverse affects on body condition, survival, or the ability to provision young, will the magnitude of any effect of the experiment be adequate to infer that observed changes in foraging effort may lead to changes in survival or reproductive success?

Response: A similar study on Antarctic fur seals has already demonstrated the value of such experimental manipulations, despite their subtlety (Boyd et al.1997). In that study, it was apparent that behavioral adjustments at the scale of individual dives allowed females in the treatment group to compensate for the additional foraging costs and that pup growth rate was less sensitive to the rate gain functions than to alterations in foraging trip duration. Similarly, we do not propose to drastically alter reproductive success, but rather to gain insight into the mechanisms that account for the natural variation we observe in the wild, so

that we can better predict the consequences of realistic changes that might be expected due to climatic change or modifications of fishing effort.

24. How reliable are the release devices that will be used for the buoyancy/drag block attachments? How soon will blocks detach if releases fail?

Response: Remote-release devices constructed by Dr. Andrews have either released on command approximately 75% of the time, and the others have broken off prematurely, so that 100% of devices were released on time or early. Nonetheless, the blocks will be attached with a back-up safety release consisting of a cable that corrodes in approximately 2 months.

25. The take tables do not appear to include disturbance and tissue collection takes by the remotely-controlled vehicle.

Response: Disturbance that is incidental to the use of the remotely-controlled vehicle are included in the incidental disturbance take category.

26. Coordination of efforts between activities proposed in this application and those by other groups is described in the application. This coordination will be most important for collection of scats from the western Gulf of Alaska and eastern Aleutian Islands with other groups (NMML, AEB, UAF, and NPMMRC) proposing collections at the same sites. Likewise, pup captures at Sugarloaf Island and Seal Rocks need to be coordinated with similar NMML activities.

Response: Substantial effort is made to coordinate all locations and times of activities with the above-mentioned agencies. All activities of Task 1 on the rookeries mentioned, as well as all other areas, will occur only after consultation with NMML and other researchers proposing to study Steller sea lions. Task 2, the Transient Project, will operate primarily in the central Gulf of Alaska. Any expansion into the western GOA and Aleutian Islands will take place with coordination efforts well in advance.

27. Are the LHX tag deployments separate from, or part of similar tag deployments proposed in application 1034-1887 by Markus Horning?

Response: The LHX tag deployments listed in this application are part of the larger scope of LHX study by Markus Horning and Jo-Ann Mellish. The tags and implantation procedures are identical. The Transient Project serves as a controlled environment for refinement of the technique (Mellish et al, in review; Thomton et al., in review). Any LHX tag deployments carried out under this ASLC application, will also count towards the sample size of 100 animals requested by application 1034-1887 by Markus Horning. See also reply to comment #16 in application 1034-1887.

28. A series of tooth measurements appear to be adequate for estimating ages of sea lions <24 months old (King et al. 2007, Marine Mammal Science 23:262-271), so there may not be a need for tooth extraction from all of the age classes requested in the application.

Response: For Task 1 we will be capturing sea lions in the > 23 months of age, so tooth extraction will provide a definitive determination of age for those sea lions. Goebel et al. (2005) examined the long and short-term effects of non-lethal tooth extraction on Antarctic fur seals, and the only short-term effect was a minor effect on maternal attendance (on-shore

visit duration was slightly longer after tooth extraction). Tooth extraction had no effect on over winter survival, fecundity, mass gain or diving behavior (Goebel et al. 2005). Tooth extraction is not requested for Task 2, the Transient Project. As we focus on juveniles (<4yrs), we are able to employ a combination of the King et al. 2007 method, adjusted for time of year.

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29. Many of the procedures that are proposed were never mentioned in the DEIS nor was their impact and/or mitigation discussed. See 50 C.F.R. § 216.34(a)(1) (the applicant must demonstrate the that activity is "humane and does not present any unnecessary risks to the health" of the animal). These include, among others: "labeled water dilution," the use of buoyancy challenge devices, use of metabolic chambers, and the use of a remote-controlled turtle-like vehicle to obtain samples and measure body condition (though we find this approach intriguing). Other instrumentation not discussed in the DEIS includes attachment of sensors to record jaw opening and closing, subcutaneous implantation of heart rate data logger, stretch sensors for measuring breathing, glued-on air-flow sensors, heat flux sensor and stomach temperature sensor "pills" whose retrieval is not discussed. These procedures, which were not discussed in the DEIS and whose effects and mitigation are not reviewed but may have a substantial negative effect on the individuals, and thus the population, should not be permitted.

Response: The use of a metabolic chamber was absent from the Appendix B (Research Methods) of the DEIS but was requested to be included in the final as per comments submitted from ASLC to PR1 on 02 April 2007. However, it does appear as a method in Chapter 2-10, 2-14, and 2-25. The use of deuterium oxide/labeled water dilution is mentioned in the DEIS in Chapter 2-9, in the Discussion of Research Components for varying alternatives. The various scientific instrumentation mentioned by the commenter are now included in Appendix B, page B-44. Stable isotope analysis is covered under the specific sample collection method requested, under section 2.19 of Appendix B of the PEIS. Deuterium dilution is covered under section 2.6 of Appendix B, administration of drugs, and section 2.11, venipuncture and blood collection. Deuterium oxide is simply heavy water, and has no known side effects. Therefore, the risk assessment associated with deuterium oxide injection and subsequent blood sampling is covered under drug injection and blood sampling, respectively. These procedures are included in the PEIS, and their effects and mitigation are described.

30. Although the applicant identifies objectives in the recovery plan, there is no attempt to provide information on hypotheses being tested or the relation of the procedures proposed to hypotheses. Discussion in the text provides vague reference to the recovery plan and NRC recommendations (e.g. page 5) but provides no specific information as to how these particular procedures or sample sizes will inform the information needs identified in the recovery plan. This is a serious omission, because NMFS may not grant a permit for research on a depleted or listed stock unless the research "fulfill[s]" an objective from the recovery plan or otherwise fulfills a critical research need. 50 C.F.R. § 216.41(b)(5)(iii). Merely mentioning recovery plan objectives without discussing how the proposed research specifically relates to an objective in the plan does not provide NMFS or the

public with sufficient information to determine whether the research will "fulfill" a research need. Id. §216.41(b)(5)(iii).

Response: The studies of Task 1 respond to multiple recommendations of the Steller sea lion Recovery Plan (NMFS 2006) and focus on action recommendation #2.3 to "insure adequate habitat and range for recovery." Our research addresses multiple sub-headings of recovery action plan #2.3, such as examining diet through scat and stomach collections (2.3.1) and fatty acid analyses (2.3.2), deploying instruments to obtain fine-scale data on foraging habitat (2.3.3) and determining foraging needs (2.3.4). We will also follow the Recovery Plan recommendations #2.4 and 2.5 to determine the environmental factors influencing Steller sea lion foraging and survival and to investigate sea lion bioenergetics. The main goal of our foraging ecology research is to address Recovery Plan recommendation #2.6.5--assess the response of sea lions to changes in prey distribution and availability. Although the current Recovery Plan is still in draft form (NMFS 2006), it should be clear that the research that we are requesting permission to conduct will fulfill some very important unmet research needs that were also identified in the previous Recovery Plan (NMFS 1992) and are still considered critical to this day. This is made clear by the following quote from the Alaska Groundfish Fisheries Draft Programmatic Supplemental Environmental Impact Statement (NMFS Sept. 2003): "The largest information gaps in understanding what has caused the decline of sea lions or preventing their recovery are in the area of nutritional stress. In particular, they involve the following issues: measuring nutritional stress in a random sample of the population; determining prey and prey field requirements to sustain healthy individual sea lions; understanding sea lion use of habitat and how this changes with age and season; discerning natural from fishery-induced changes in the prey field." Our research will directly examine the nutritional and prey field needs of Steller sea lions, and it will also determine how variation, in space and in time, in the prey field affects the health and reproductive success of Steller sea lions. Therefore, this research will contribute in a very significant way to conservation efforts.

The specific objectives as outlined in the original application and related multiple hypotheses with applications to Recovery Plan criteria for the Transient Project, Task 2, are as follows:

Objective 1. Ongoing collection of baseline health parameters of pups (6-11mo) and juveniles (12-48mo)

H_o: General health and body condition in the central GOA and Resurrection Bay area are within normal parameters

H_o: General health and body condition of individuals collected for temporary captivity for research purposes are within current normal expected values for the local population

Recovery Plan Applications (1.3, 2.1, 4.1, 4.2) - Continued monitoring of the general health, body condition, contaminants levels of endangered Steller sea lion populations was listed as a priority, in particular to investigate the effects of season, age and sex. Access to young of the year and juveniles through the Transient Project will help to address this need, as we collect throughout the year (1.3.1). In addition, we have the ability to help develop a comprehensive index of health (1.3.2), as we can provide multiple concurrent samples of hematology, chemistry, immunology and endocrinology from collaborating investigators, with the unique added ability to collect samples over a longer timeframe (up to 3 months) in given individuals with detailed dietary and physiological history.

Objective 2. Temporary captivity for research purposes for up to 30 animals/year

H_o: Juvenile Steller sea lions can be successfully maintained in temporary captivity for research purposes, to provide a collaborative platform for comprehensive study of wild individuals in controlled environment
Recovery Plan Applications (5.6) – While this objective does not address specific physiological hypotheses, it is essential to the completion of the majority of the Transient Project, with the exception of free-range control sampling. The physical location of the facility adjoining the ASLC provides a strong public education resource. Interpreters at the ALSC are able to educate the visitors, schoolchildren and other outside groups as to the status and biology of the Steller sea lion, as well as the specific research we are conducting at the facility to address the Recovery Actions for the population.

Objective 3. Continue post-release monitoring via visual resight (e.g., hot-brand), scientific tag attachment (e.g., satellite tag, video data recorder), and LHX implantation
H_o: Dive and ranging behavior of individuals handled for research purposes, including temporary captivity for up to 3 months, will not differ significantly from wild conspecifics.
H_o: Instrumented juvenile sea lions forage primarily within existing critical habitat designated areas
H_o: LHX implant recipients do not differ in post-release behavior compared to non-implant recipients and wild conspecifics
H_o: Juvenile survival rates do not differ from mathematical estimates (e.g., York 1994)
Recovery Plan Applications (1.2, 2.1, 2.2, 2.3, 2.4, 5.3) - This objective addresses many issues ranging from basic survival rates of juveniles (via LHX implants), an ongoing need to observe dive behavior and ranging location for critical habitat designation and to enable the monitoring of animals post-release for the essential assessment of the longer-term impact of our research activities.

Objective 4. Validate the use of non-invasive tools (e.g., thermal imaging, ultrasound, 3D imaging) for the determination of health and condition indices
H_o: Heat loss/ thermal signatures of Steller sea lions can be identified via thermal imaging technology
H_o: Blubber depth via imaging ultrasound corresponds to patterns of heat flux/ thermal signatures of Steller sea lions
H_o: Thermal signatures can be used as a remote proxy for body condition
Recovery Plan Applications (1.3, 1.4) – There is increasing demand for non-invasive technology capable of providing physiological information. Two particularly promising techniques include the use of imaging ultrasound and thermal imaging. Imaging ultrasound to measure site-specific blubber depth (Mellish et al. 2004, Mellish et al. 2007) and reproductive status (Adams et al. 2007) in otariids has been validated. However, it still requires physical contact with the animal. Thermal imaging has been used in a preliminary fashion to assess heat flux in sea lions (Willis et al.,), and with continued testing in controlled conditions may provide a useful resource for remotely determining overall condition, heat loss, regional blubber depth, and wound identification.

Objective 5. Perform calibration studies for nutritional baseline analyses (e.g., stable isotopes, fasting metabolites)

H_o: Stable isotope signatures in Steller sea lion tissues reflect dietary patterns in a predictable fashion
H_o: Stable isotopes can be used as an effective tool define foraging ecology in Steller sea lion tissues
H_o: Fasting metabolites change in a predictable fashion according to nutritional state of juvenile Steller sea lions
H_o: Fasting metabolites can be used as an accurate indicator of nutritional/fasting status of juvenile Steller sea lions
Recovery Plan Application (1.3, 2.3, 2.5) - As discussed in detail in the Recovery Plan, traditional fieldwork provides a single snapshot of an individual's health. Controlled experiments that simulate a range of dietary and nutritional states can provide a critical baseline and standard by which to assess field-collected samples. Both stable isotope methodology and fasting metabolites were specifically identified as potential tools for a more accurate assessment of individual health.

Objective 6. Examine physiological response to restricted intake/fasting via metabolic chamber, body condition assessment (ultrasound, d2o) and heat flux
H_o: Metabolic rate, total body fat and blubber depth will decrease with restricted dietary intake/fasting
H_o: Total heat loss assessed via thermal imaging will increase as individuals lose insulatory blubber reserves
H_o: Regional heat loss will increase with decreased blubber depth
Recovery Plan Application (1.3, 2.3) – The understanding of an individual's response to nutritional stress is essential to the overall interpretation of health in the context of their environment. In conjunction with Objective 5, we will test the specific energetic responses to a spectrum of restricted intake through complete fasting using a suite of complementary tools. Metabolic rate assessments will allow for the calculation of daily energetic requirements. Body condition measured through deuterium oxide dilution and imaging ultrasound will provide overall and site-specific energy depletion. Heat flux/infrared thermography will provide a non-invasive assessment of overall and site-specific heat loss. Extended studies such as these are only capable in controlled, captive settings, yet they provide essential baseline data for field comparisons.

Objective 7. Study foraging behavior and habitat selection through scientific instrument attachment
H_o: Instrumented juvenile sea lions forage primarily within existing critical habitat designated areas
Recovery Plan Applications (2.3, 5.3) – In conjunction with Objective 2, this portion of the Transient Project addresses an ongoing need to observe dive behavior and ranging location for critical habitat designation. Information gathered from these individuals can be compared to previously collected data to determine any shift in foraging patterns or locations with potential management implications. Due to the location of the Project, we are in some instances able to recover instruments that transmit from land locations, with the potential for necropsy if the tag is beached due to mortality of the individual.

31. Both this applicant and Horning propose to implant life history (LHX) transmitters. Though Horning states that a portion of his sample size may be met with animals proposed under the ASLC proposal, this permittee does not acknowledge Horning or the relationship of their activities to his proposal. If NMFS grants a permit for this activity (and we do not believe it should) then this applicant's proposal should be subsumed by Horning and not granted separately. 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"). This assures a means of limiting effects and also assures that the multiplicity of procedures proposed by this applicant are not added to surgically challenged animals used in the LHX study. Horning stated that the purpose of the study was to monitor behavior of animals. We believe that capture and holding of animals for weeks at a time, subjecting them to anesthesia, invasive procedures and altered diet (as well as possible additional instrumentation) may compromise the validity of data on foraging and other daily behaviors as animals re-acclimate to the wild and forage naturally after their recovery.

Response: Markus Horning is listed as a Co-Investigator on application 881-1890, and Jo-Ann Mellish is the Co-Investigator for the project in application 1034-1887. The Transient Project is ideal for the initial stages of the LHX project, with a quarantine environment and controlled setting for extended monitoring. The temporary captivity has been shown to have no substantial physiological effects on general health (Mellish et al. 2006), and no lasting influence on post-release dive behavior of both non-implant individuals and implant recipients (Thomton et al., in review a,b). To the contrary, both groups of animals, including the initial set of 6 implant recipients, were performing within normal dive duration, depth and location parameters within days of release (described in detail in response #46). We feel that continued monitoring of implant recipients at the quarantine facility can only improve the understanding of the procedure, as well as to provide a crucial monitoring program for the temporarily captive animals. Few individuals in the population will be as well studied such that we will have detailed health information at the time of implant to compare to survival parameters.

See also reply to comments #1 and #16 in application 1034-1887 (M. Horning)

32. In all permit applications involving the transport and captivity of threatened or endangered species, the MMPA's implementing regulations require specific information to be in permit applications that does not appear in this application. For example, the permit must supply the name and "qualifications" of the transport company, the length of time in transit, a description of the pen or container at capture cite and during transport, a statement whether an vet or other qualified person will be there and a description of why that person is qualified, and specifications about care (dimensions of the pool the animals will be held in, the amount and quality of the water, the diet, sanitation, and qualifications of the staff), and a "certification" from a vet or recognized expert saying the transport/holding will be adequate 50 C.F.R. § 222.308(7), (8). These required assurances should have been, but were not, provided as part of this application which seeks to capture animals and move them to the ASLC facility.

Response: Transport and care of all animals under the Transient Project is provided by experienced Research, Mammalogist and Veterinary Staff members. Details of the facility

are described in Mellish et al. 2006, as referenced in the permit application. The facility has been inspected and licensed by USDA. Copies of the current license and most recent inspection report have been provided to PR1, along with a letter of certification from Pam Tuomi, DVM. Prior to all transport activities, a detailed transport plan is filed and approved by the ASLC IACUC, with specific details of the transport mode, enclosures and personnel.

33. Task 1 under this permit is the study of free-ranging Steller sea lions from the Western DPS. This would affect up to 610 animals (page 2). No justification was given for the sample size nor do the summary charts appear to substantiate this number. This should be clarified.

Response: For each age class we are proposing to capture between 40 and 80 individuals. We have conducted similar studies in the past (Andrews 1998, 2004; Andrews et al. 2002) and these have provided us with an estimate of the amount of variation that we can expect in many of the parameters that we plan to measure. Power analysis has demonstrated that for many of the variables we will need even larger sample sizes than the ones we have proposed in order to detect slight differences in response to the anticipated variation in prey availability within one year. However, because the capture and instrumentation of Steller sea lions is logistically difficult and very expensive, we hope that even with a limited sample size that we will gain extremely valuable data and insights. For adequately describing habitat use, a sample size of 40 should be adequate, based on on a study in which southern elephant seals were instrumented with satellite tracking devices (Hindell et al. 2003). That study demonstrated that at least 25 animals were necessary to provide a useful representation of habitat use, but that nearly 95% of the actual area used would be identified with a sample of 40 individuals.

34. Although the text states that work will focus on maternal behavior and physiology (page 3) the summary charts do not indicate a differential focus on females.

Response: Task 1 will investigate both adult females and juveniles as explained in the application. For example, on page 4 we state: We plan to address this critical information gap by monitoring the behavior of adult female Steller sea lions at sea and on land and exploiting temporal and spatial variation in prey bases and population trends. Because low juvenile survival was a problem in the past and because low fecundity may result from inadequate maternal provisioning during developmental stages, we will also study pups from birth through sexual maturation.

35. Darting with Telazol is inappropriate. The NMML provided a chart and information showing that, although documented deaths from darting were low, a high percentage of animals either went into the water or to inaccessible areas making it impossible to monitor their fate. Deaths have been documented. There are additional concerns with the use of Telazol, which does not (according to the DEIS) have a reliable antidote. See 50 C.F.R. § 216.34(a)(1) (the applicant must demonstrate the that activity is "humane and does not present any unnecessary risks to the health" of the animal).

Response: *We recognize the risks associated with the use of Telazol and will only dart adult females on a rookery when the behavior of the female makes it much less likely that she will enter the water than is the case for other age classes or at other times of the year.*

36. The studies cited on page 14 for impacts of branding are incomplete and omit mention of studies such as Scordino (2006), who found an increased death rate in branded pups.

Response: We were unaware of the completion of the Scordino (2006) thesis at the time our application was prepared. Scordino clearly stated in the conclusion that all estimates were based on "apparent survival" and were confounded by emigration. Other than this recently completed but unpublished thesis, the Final EIS does a thorough job of reviewing the literature relevant to branding.

37. Task 2 also studies free-ranging animals. Of these animals, up to 30 may be held captive for up to 3 months (see comments on Task 3 below). Free-ranging animals will be subjected to attachment of various scientific instruments, though the combination that will be used is not clear either in the text or the summary charts. A variety of instruments are proposed, including satellite-linked dive recorders and 5 juveniles will have video system data loggers and data transmitters. The applicant must make clear which combination of instruments are proposed for attachment so that NMFS and external reviewers can be assured that the combination is appropriate for undertaking the proposed investigation and that they will not unduly compromise the animal. The MMPA's regulations expressly require that each permit application provide a "description of the manner of taking for each animal, including the gear to be used." 50 C.F.R. § 222.308(b)(6)(i) and, although a variety of instruments are described, the combination of their use (and thus the hypothesis being addressed and the relative risk to animals) are not specified.

Response: At release, each individual that undergoes temporary captivity may be subject to a combination of up to 2 intra-abdominal data loggers (LHX) to record survival and long term behavior, and one external satellite-linked data logger/transmitter to record and transmit short-term behavior (< 5months) post-release. Of the 30 individuals, up to 5 may have a third instrument attachment, the VDAP recorder. This instrument is also a temporary attachment and will be recovered via remote-release.

38. Anesthesia is only administered to sampled animals "if deemed necessary by the attending veterinarian." (page 39) This is not appropriate. Analgesic should be provided to any animals subjected to painful or/and stressful procedures. The applicant proposes to withhold food for 12 hours as a safety precaution, but only for captive animals. The rationale should be provided for the differential safety risk to wild and captive animals such that this is necessary for only one of the two groups.

Response: Food is withheld from temporarily captive animals for 12 hours prior to anesthesia as an added safety measure, due to the benefit of the controlled setting. Animals in the field are processed immediately, as we do not have the desire or capacity to hold them without food for 12 hours. The differential treatment is not a function of reduced care to either group, but a logistical consideration.

39. Page 22 lists objectives for the program. One of them (#2) is "temporary captivity for up to 30 animals/year." This is a method, not an objective. Or at least it shouldn't be an objective.

Response: Temporary captivity is listed as the primary objective, as the method itself is the platform for the subsequently proposed research.

40. The discussion of scientific instrumentation on pages 30-32 details a number of instruments that can be attached to juvenile Steller sea lions in various combinations. These include: data loggers to record depth swim speed and acceleration (attached at 3 points on the animal), digital camera, video camera, sensors for jaw opening, stomach temperature sensor "pills," subcutaneously attached heart rate logger, straps around the chest to measure breathing, air flow sensor, heat sensor and buoyancy "challenges." The applicant stresses on page 32 that no animal will receive more than a head-mounted instrument, a mid-dorsum mounted instrument package, a stomach temperature "pill" and a third package of a satellite transmitter and VHF instrument package glued to the fur. In other words, a single animal can be subjected to the insertion or attachment of 5 instruments. The applicants state that they "will determine the exact combination of instruments depending on the age and size of the sea lion, the season, the location, whether simultaneous fish assessments are occurring in the area, and whether the sea lion "will be under simultaneous visual observation." This latter criterion is not explained (i.e., how visual observation will facilitate the attachment of some instruments but not others).

Response: Sea lions that are captured at the Chiswell Island rookery can be remotely-monitored using our remote-video monitoring system. Because we can use the video cameras to visually monitor instrumented sea lions on land, we can better ascertain the effects of instruments on certain parameters of reproductive performance, such as time spent resting and time spent nursing the pup. Therefore, larger instrument packages, such as the VDAP system will be preferentially attached to sea lions that can be monitored visually in this way.

41. Nor does this application meet the requirement to describe manner of taking "each" animal, "including the gear to be used." 50 C.F.R. § 222.308(b)(6)(i). The applicant must be more specific about the criteria it will use for determining which instrument or combination of instruments will be chosen. Clearly these instruments are for different purposes. There is no explanation of the procedures sufficient to determine whether a variable number and combination of devices will yield sufficient information of sufficient quantity or quality to inform a significant hypothesis regarding nutritional stress. That is, if an animal has a head-mounted jaw opening sensor attached to its head, breathing sensor straps on its midsection, a stomach temperature sensor, and a VHF and satellite transmitter pack; how will that relate to data from a different animal that may have a head mounted digital camera, a heart rate logger, a stomach temperature sensor and a VHF transmitter pack or another animal that may have a head mounted jaw opening sensor, a heart rate monitor, a stomach temperature sensor and the VHF package?

    Since instrumentation will vary (and may or may not include the buoyancy challenge devices that are described in the application) how will data from various combinations of instrumentation be integrated and/or provide a robust sampling?

Response: The different sensors that we propose to use do serve our purpose of determining how variation in prey type, abundance and distribution affect the behavior, condition, survival and reproductive success of individual sea lions. The different instruments are just complementary ways to measure energy output (heart rate, breathing rate, heat flow,

acceleration and movement) and energy input (jaw opening sensor, stomach temperature, imaging of ingested prey with CCD). A single instrument can have a sensor for dive depth, acceleration, jaw opening, and have a receiver for the stomach temperature transmitter as well as be satellite-linked. In our permit application we have provided an indication of the possible combinations of sensors and instruments that would be attached to a sea lion. The data from each sensor is integrated to provide an estimate of these two parameters for each instrumented sea lion: energy output and energy intake. In order to achieve a positive nutritional balance, the energetic costs of acquiring prey must be less than the energy obtained from the prey. To fully examine the factors that affect the population status of Steller sea lions, one should quantify both sea lion energy expenditure and prey ingestion. This is especially true when it is necessary to determine how spatial and temporal variation in the prey field affects health and reproductive success.

42. With regard to the buoyancy challenge (which was not assessed in the DEIS) will animals also have camera packages attached in addition to the dive behavior logger and "blocks" that are attached for this experiment?

Response: *The use of devices to alter buoyancy or drag is now mentioned in Appendix B of the Final EIS, on page B-44. Sea lions that are instrumented with camera packages will not have buoyancy or drag altering blocks applied.*

43. How can the applicant assure that the various combinations of procedures will not have adverse cumulative or synergistic effects on the animals?

Response: All captured sea lions will be branded so that we will be able to assess both the short-term effects and the long-term effects (on survival and reproduction) of these manipulations.

44. Further, the sample sizes described in the text on page 33 do not appear to fully comport with the summary charts provided at the end of the application. The applicant should check to assure that sample sizes in both places are the same and have a scientifically determined basis.

Response: We have checked and the sample sizes are consistent. Those samples mentioned on page 33 are subsets of the total number of sea lions that will be captured and instrumented with scientific instruments.

45. This proposal would also subject animals to bioelectric impedance analysis (BIA). Other applicants (e.g. ADFG) have stated that this needs to be done in conjunction with administration of deuterium oxide dilution. If this is correct, then the BIA requested in this permit should not be granted since the used of deuterium oxide was not analyzed in the DEIS and thus should not be permitted.

Response: BIA does not have to be done in conjunction with deuterium oxide. In species for which there is no published data on the relationship of the reactance and resistance values obtained by BIA, then one must first conduct a study in which total body water is measured by labelled water dilution and the correlated with BIA values. However, we have access to such data from a study that has been completed for Steller sea lions (M.C. Castellini, unpublished data).

46. Task 3 involves the capture of up to 30 juvenile (1-4 year old) Steller sea lions to be held captive for up to 3 months for the purpose of multiple sampling procedures and forced dietary changes. We would have appreciated a discussion of the known post-release fate of animals previously subjected to these sorts of experiments by the applicant and what percentage were not re-sighted.

Response: An in-depth study of the post-release behavior of all juveniles through the transient program to date (including LHX implant recipients) is currently in review (Thomton et al., in review a,b). A brief discussion of the relevant findings for all animals, post-release is included below. However, it should be noted that all animals, including those with dietary challenges, must be within acceptable mass (e.g., within 15% of entry mass) and body fat (10-25% total body fat) ranges prior to release.

Juvenile Steller sea lions released from the temporarily captive facility were tracked with externally attached satellite-linked data recorders for 74.1 ± 9.6 days (range 14 - 160 days). The mean dive depth (26.2 ± 4.0 m), dive duration (1.4 ± 0.1 min), dive rate (10.1 ± 0.5 dives hr-1), trip duration (14.1 ± 2.1 hr), haul-out duration (11.2 ± 1.5 hr) and time wet (46.9 ± 2.6%) were within the range of previously published values. Movement (190.0 ± 31.9 km) between haul-outs and rookeries during the tracking period was also typical of juvenile Steller sea lions in Alaska. Following release, juvenile Steller sea lions performed shorter and shallower dives during the first week post-release, than free-ranging conspecifics. However, all parameters were within expected ranges for juveniles of the same population by the second week post-release. The overall mean dive depth, dive duration, trip duration, haul-out length, dive rate, percentage of time wet, and dispersal were equal to or greater than the FR control and published juvenile SSL values. Furthermore, during the 2.7 mos tracking period, TJs appear to equal adult female diving abilities. The results of this study indicate that temporary captivity of up to three months probably does not impair dive performance or dispersal. In addition, dive development may continue until at least 24 mos of age.

LHX implant recipients - During the 91.5 ± 8.6 day tracking period following release from temporary captivity, all sea lions returned to their respective capture haul-outs. Dive depth, duration, frequency and dispersal distances were similar to non-implanted animals. A possible captive recovery effect was observed during the first week post-release when dive depths were shallower and durations shorter than free-ranging juveniles. By the second week all dive behavior parameters were within the normal range and did not differ from non-implanted animals. This effect was also observed in non-implanted individuals maintained in temporary captivity. In conclusion, surgical implantation of LHX tags does not appear to have a negative effect on the short-term dive behavior or movement of juvenile sea lions. See also response to comment #1 in application 1034-1887 (M. Horning).

47. We also wish to point out that this application more than doubles the number of animals previously permitted for this type of study. We see no evidence that the applicant institution's facility has been enlarged to accommodate this activity and that of their other permit request to captive-breed Steller sea lions (file #881-1745). Although it is up to APHIS to determine suitability of housing for captive animals, the MMPA regulations require the permit application to describe the containment facility in detail and provide a certification from a licensed veterinarian or other expert that the facility is adequate to provide for the animals well-being. 50 C.F.R. § 222.308(8).

Response: The quarantine housing facility as designed and constructed for the Transient Project is capable of housing up to 6 juvenile males or 11 juvenile/adult female Steller sea lions as per USDA and APHIS regulations. As in response #32, the facility has been inspected and licensed by USDA. An additional letter of certification was provided at the time of application by Dr. Pam Tuomi, DVM. The facility is described in detail in Mellish et al. 2006.

48. The applicant should supply hypotheses being tested as other permit applicants have done and should clearly relate procedures to the hypothesis being investigated (e.g., animals with slower rates of breathing and lower body temperature are more or less likely to forage effectively, or animals diving to specific depths over specific time periods are more or less likely to be effective in foraging). Given the large number of novel procedures being proposed, and the multiplicity of devices proposed for attachment to animals, there should be a justification for them and none is provided other than the vague assurance that they relate in some unspecified manner to the investigation of nutritional stress as a contributor to ongoing declines.

Response: As is explained in the application, the various sensors that the commenter mentions are just different ways to measure the cost and benefits of foraging. We provide very specific hypotheses and justification for studying the foraging behavior and ecology of Steller sea lions in the application. This concern is also discussed in detail in response #30.

Literature cited

Adams GP, Testa JW, Goertz CE, Ream RR, Sterling JT (2007) Ultrasonographic characterization of reproductive anatomy and early embryonic detection in the northern fur seal (Callorhinus ursinus) in the field. Marine Mammal Science 23: 445–452.

Andrews RD (1998) Remotely releasable instruments for monitoring the foraging behaviour of pinnipeds. Marine Ecology Progress Series 175: 289-294.

Andrews RD, Calkins DG, Davis RW, Norcross BL, Peijnenberg K, Trites AW (2002) Foraging behavior and energetics of adult female Steller sea lions. In: Steller sea lion decline: Is it food II, ed. D. DeMaster and S. Atkinson, pp. 19 – 22. University of Alaska Sea Grant, Fairbanks.

Andrews RD(2004) The population decline of Steller sea lions: testing the nutritional stress hypothesis. In: Experimental approaches to conservation biology, (ed. M. Gordon and S. Bartol). Berkeley: University of California Press, pg. 132-146.

Boyd IL, McCaferty DJ, Walker TR (1997) Variation in foraging effort by lactating Antarctic fur seals: response to simulated increased foraging costs. Behaioral Ecology and Sociobiology 40:135-144.

Costa DP, Gales NJ, Goebel ME (2001) Aerobic dive limit: how often does it occur in nature? Comparative Biochemistry and Physiology A 129:771-783.

Garza D, Becan-McBride K (1984) Phlebotomy handbook. Prentice-Hall, CT. 305 p.

Hindell MA, Bradshaw CJ, Sumner MD, Michael KJ, Burton HR (2003) Dispersal of female southern elephant seals and their prey consumption during the austral summer: relevance to management and oceanographic zones. Journal of Animal Ecology 40:703–715.

Mellish J, Horning M, York A (2007) Seasonal and spatial blubber depth changes in captive harbor seals (Phoca vitulina) and Steller's sea lions (Eumetopias jubatus). Journal of Mammalogy 88: 408-411.

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

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

Mellish J, Thomton J, Horning M (in review) Physiological response to intra-abdominal satellite transmitter implantation in endangered Steller sea lions, Eumetopias jubatus. Journal of Experimental Marine Biology and Ecology.

Richmond JP, Burns JM, Rea LD (2006) Ontogeny of total body oxygen stores and aerobic dive potential in Steller sea lions (Eumetopias jubatus). Journal of Comparative Physiology B 176: 535-545.

Thomton J, Mellish J, Horning M. (in review, a) Dive behavior and movement patterns of juvenile Steller sea lions after intra-abdominal transmitter implantation. Journal of Experimental Marine Biology and Ecology.

Thomton J, Mellish J, Horning M. (in review, b) Effects of temporary captivity on diving and ranging behavior of juvenile Steller sea lions, Eumetopias jubatus. Marine Ecology Progress Series.

Comments received on File No. 434-1892 (Oregon Dept. Fish and Wildlife)

Marine Mammal Commission (MMC)

1. The application does not, but should, indicate the minimum age at which pups would be captured, branded, instrumented with VHF transmitters, etc.

It is not possible to determine the age of pups with certainty. However, we handle pups no earlier than the second week of July (following the end of the pupping period) at which time pups range in age from approximately two weeks to two months. Furthermore, we do not conduct research on pups weighing under 20 kg or that have an attached umbilicus.

2. Page 14 of the application states that "[a]dult sea lions are branded without anesthesia because they are able to be restrained more efficiently and safely using the squeeze cage." As noted above, we recommend that the Service require the applicant to provide further justification for proposing not to anesthetize adult animals during branding prior to issuing authorization for such activities.

We no longer plan to study adult Steller sea lions and therefore withdraw all requests related to their take.

3. The application states that the Oregon Department of Fish and Wildlife does not have an Institutional Animal Care and Use Committee and that "the Animal Welfare Act does not apply in this case." The Animal and Plant Health Inspection Service (APHIS), the agency responsible for implementing the Animal Welfare Act, has advised the Service that the applicable regulations require facilities that use live animals for research or experimentation to establish and use IACUCs. This includes not only research in captive settings but also field studies involving invasive procedures or those that harm or materially alter the behavior of the animals being studied. The Service should defer approval of the application until the applicant provides documentation that the proposed research has been reviewed and approved by an IACUC in accordance with § 2.31 of the Animal and Plant Health Inspection Service's regulations implementing the Animal Welfare Act. As for the claim by the applicant that the IACUC requirements do not apply to its activities, the Marine Mammal Commission recommends that the applicant be provided with a copy of our 17 January 2007 letter concerning IACUCs and be referred to the Animal and Plant Health Inspection Service to clarify the situation.

The Oregon Department of Fish & Wildlife is mandated by state law to conduct research and management activities with many species of fish and wildlife. We have not had, and currently do not have, an internal IACUC. However, our staff veterinarian, Dr. Colin Gillin, provides oversight on agency activities involving live animals, including the research described in our current permit application. Furthermore Dr. Gillin is an active participant in our research (see next comment). It is our understanding that due to the urging of the Marine Mammal Commission, the NMFS is currently considering

developing policy which might require IAUCC review for all but observational studies covered by MMPA and ESA Research Permits but until that time it will not be part of the permitting process.

4. It is unclear whether a veterinarian will be present in the field to oversee branding and other invasive activities. Clarification of this point should be provided. If a veterinarian will not be present in the field, an explanation should be provided. If a veterinarian will be present, his or her curriculum vitae should be submitted if it is not already on file.

While handling pups, we typically have from 2-4 veterinarians and an additional 1-2 vet technicians on the study site at all times. These persons operate the gas anesthesia equipment and respond to any emergency situations that might arise. We have attached the curriculum vitae of our agency veterinarian Dr. Colin Gillin; the CV of Dr. Francis Gulland, who usually participates in our work is already on file.

5. In discussing the floating platform method of capture, the application states that one to ten sea lions may be present when the door is closed. The application also should state the maximum time animals would be maintained in the holding cage before they are sampled or released.

We no longer plan to study <u>adult</u> Steller sea lions and therefore withdraw all requests related to their take.

6. Additional information should be provided concerning the proposed short- and long-term monitoring of animals to allow reviewers to evaluate whether monitoring will be sufficient to assess the effects of branding/handling on the subject animals.

All pups are observed closely after gas anesthesia to ensure full recovery prior to release. Animals are held in a protected location on the rookery during recovery under the protection of researchers until they regain mobility and move away under their own power. Pups are then monitored in the short-term on the rookeries via remotely operated video cameras and during vessel surveys and counts. In the long-term, year-round efforts to re-sight marked animals occur at many locations in the study area. Digital images of brands are taken at the time of marking and as often as possible during re-sight efforts. Conditions of brands are recorded and described during each re-sight event in subsequent years.

<u>USDA Animal and Plant Health Inspection Service (APHIS)</u>

7. While there may be reasons to use hot branding for permanent identification, the activities in the multiple Stellar [sic] sea lion and northern fur seal applications do not warrant its use. The study design does not require and does not benefit from capture/release/recapture. All biochemical and body morphology do not require multi-year sampling of identified individuals. Any identification needed in one

year to prevent resampling of the same animals can be served well by bleach or paint.

Branding should not be used unless it is the only means of identification possible. Since the protocols do not call for monitoring specific individuals as a key component, hot branding is not acceptable for these permits.

PR1: Please explain why your study cannot be accomplished without hot brands.

The reviewer clearly misunderstood the nature of the proposed research. The primary reason for branding pups is demography which requires an identifiable individual mark throughout the lifetime of an animal. This is required to estimate age specific survival, natality, recruitment and senescence which have been identified as essential information by the Federal Steller Sea Lion Recovery Team for addressing the recovery of Steller sea lions. Hot-branding is the only currently known way to permanently mark pinnipeds with individual marks that can be easily read from a distance, over the lifetime of the animal (20+ years). Flipper tags and other marking methods are not acceptable for estimating vital rates of Steller sea lion populations as they are either short-term or unreadable at a distance.

8. In addition, under the AWA, any time a potentially painful or distressful procedure is to be used, alternatives must be researched and final choices must be justified for the study. In this consideration of alternatives, reduction of numbers, refinement of technique, and replacement with other animals must be considered. If a study exists that requires hot branding (not considered humane), the length of time the branding takes, post procedure treatment and alleviation of pain, and other options must be considered. In the studies under review, there is no justification for using 4 digits, or for branding each digit alone.

   At a minimum, 3 digit branding is sufficient to mark more animals that needed for the studies (34X34X34 – over 39,000 combinations, using only 0 and 1, not "o" and "I"). Additionally, devices should be used that load all three irons together so that only one application of the brand is used (20 sec as opposed to 2 minutes).

   PR1: Please explain the need for 4 digit brands, i.e., why aren't 3 digit brands used. Please also explain why each digit is applied separately.

Four digits are currently required to indicate number 100-999 and natal rookery (letter; e.g., R, Y, F, etc.). Obviously, only two brands were required initially (1-9), and then three (10-99); eventually, five digits may be required if sample sizes exceed 999. All researchers use this system throughout the range of species' range. The reviewers comment regarding "34X34X34" combinations was unintelligible.

It would not be possible to apply more than one mark (number/letter) at a time. Each digit is carefully applied on a specific spot with extreme care and attention to pressure

and time of application. Since the animal's body is not flat, a device designed to place all marks at once would remove the ability to monitor each mark and would result in unacceptable variability in the quality of the mark. In addition, a bar upon which 4 digits are mounted will accumulate too much heat and lead to over-branding. We have communally thought of these things but ultimately rejected the idea in favor of single digit application.

9. No consideration has been made for post procedure treatment with antibiotics or pain relief has been addressed. All facilities would be required to have an approved protocol from their IACUC that has shown consideration of alternatives and use of methods that would alleviate discomfort, stress, and long-term complications. Topical antibiotic/anesthetic creme should be used post procedure.

   PR1: Please explain what considerations have been made for post-procedure analgesia and antibiotics.

We will consult with our agency veterinarian and others to evaluate and determine the efficacy of a post-branding application of antibiotic/pain relief treatment.

10. No consideration was given to proper recovery times for anesthesia. While an animal may be awake and mobile within 20 minutes of cessation of isoflurane, it is a recognized occurrence in veterinary medicine that the effects of anesthesia do not dissipate after 20 minutes. In practice, we have seen animals take up to 24-48 hours to recover from anesthesia, especially when the procedure of lengthy (over an hour).

    PR1: Please explain what considerations are made for recovery of animals from anesthesia versus the initial return to consciousness.

All sea lion pups that are anesthetized are released back onto the center of the rookery, away from the water, where they rejoin the other pups and remain until research activities have ended at the end of the day. The duration of anesthesia from cone on to cone off averaged 8 minutes for pup branding on a sample of 160 pups in 2005. Recovery from these short anesthesia are quick and considered to be complete. The reference to long duration anesthesia ("over an hour") in the above comment is simply not relevant to the pup branding.

11. As discussed above, all should address the potentially painful procedures and care of the animals.

    PR1: Please explain measures that would be taken during and after potentially painful procedures.

Pups are anesthetized during the handling and marking process thereby eliminating stress and any pain from the application of the brand. All sea lion pups that are anesthetized are released back onto the center of the rookery away from the water. They are observed

until they can move away on their own to rejoin the other pups on the rookery and remain in those pup aggregations until research activities have ended at the end of the day.

NMFS Reviewers

12. The applicant should coordinate with the Southwest Region (SWR) regarding tag color and tag type used in northern California. The applicant should notify the SWR approximately 2 weeks prior to beginning research activities in northern California. The applicant should provide an annual report to the SWR detailing research activities and listing the range of tag numbers used in northern California.

We will comply with reviewer's request for coordination when working in northern California.

The Humane Society of the United States (HSUS)

13. The Marine Mammal Protection Act (MMPA) mandates that scientific research be humane. 16 U.S.C. § 1374(b)(2)(B); 50 C.F.R. § 216.3 (a)(1). The MMPA defines the term "humane" in 16 U.S.C.§ 1362(4) as: a "method of taking which involves the least possible degree of pain and suffering practicable to the mammal involved." If researcher chooses to use a more invasive or risk-prone technique than one that fills the same purpose and need but is more risk-averse or less invasive, this research cannot be considered adequately humane. We note that although there are more risk-averse approaches available, some permit applicants have chosen more risk-prone approaches.

    For example, whereas the DEIS discusses the effective use of aerial photogrammetry to count pinnipeds, stipulating that it was found to be as reliable as the more risky use of drive counts, not all applicants wish to use it. For example, while Dr. Wynne (File No. 1049-1866) proposes to use this for her studies of population abundance and trends, other permittees (e.g. File No. 434-1892) do not and instead wish to rely solely on drive counts that are demonstrably more likely to cause disturbance, injury and risk of death than aerial survey approaches which are used successfully with pinnipeds in most areas of the U.S.

    Further while some applicants (e.g., Trites and Wynne) propose to collect scat to investigate nutritional stress and seasonal variations in diet, other permit applicants propose invasive sampling of the digestive tract (e.g. enemas, stomach intubation, fecal loops) without discussion of why a less invasive approach such as scat analysis is not appropriate. If these other studies are justified, reasoning should be made clear and there should be a discussion of how their results will be integrated into the studies of scat analysis to provide a holistic picture.

    The NMFS should not permit the use of the most risk-prone techniques where there are clearly more risk-averse methodologies available. 50 C.F.R. §

216.34(a)(1) (the applicant must demonstrate that the activity is "humane and does not present any unnecessary risks to the health" of the animal).

The reviewer states that "…other permittees (e.g. File No. 434-1892) do not [use aerial photogrammetry] and instead wish to rely solely [emphasis added] on drive counts…". Section IV.C.2.b. of our application clearly states that we will conduct aerial surveys in addition to ground counts (not "drive" counts) to assess population trends. What is not clearly stated in our application is that aerial surveys are the primary means by which we assess population status and trend of non-pups; ground counts are only occasionally used to obtain counts of pups which are much more accurate that the aerial survey counts of pups. Further, the reviewer fails to recognize that substrate differences between rookeries in Alaska and Oregon can affect the ability to accurately interpret aerial imagery when counting pups. Lastly, we make ground counts of pups in coordination with other activities conducted on the rookeries (e.g. remote camera maintenance, scat collections, marking and sampling activities); we do not typically conduct ground counts as a stand-alone activity.

The reviewer states that "…other permit applicants propose invasive sampling of the digestive tract (e.g. enemas, stomach intubation, fecal loops) without discussion of why a less invasive approach such as scat analysis is not appropriate." Again, this does not accurately reflect what we have proposed. Our use of fecal loops is to sample for the prevalence of parasites within the population, not for food habits analysis. We too rely solely on scat collections for food habits analysis.

14. This permit is noticeably brief considering the scope of activities proposed. It mentions general goals in the recovery plan that it feels the research will address but provides no specific hypotheses or variables being tested. Instead it states in the most general of terms on pages 8-9 that the applicant seeks to "provide information" on vital rates. See 50 C.F.R. § 222.308(b)(4), (b)(5) (requiring applicant to provide a "detailed" description of the project and the need for the project).

The following is excerpted from pages 8-9 of our application. In it we state the specific goals of the recovery plan (and other efforts under the MMPA) our research addresses; we have added formal hypotheses at the request of the reviewer.

The proposed activities in this permit request would build upon past research including over 20 years of aerial survey data on Steller sea lion distribution and abundance in the southern extent of the eastern DPS (Fishery Bulletin 107:102-115; ODFW unpublished data), and 5 years of survival data (ODFW and NMFS unpublished data). The continuation of these activities is necessary in order to acquire a standardized, robust, time-series of data for understanding long-term population variability and detecting changes in population status and health. These data are essential for stock assessment reports, making required potential biological removal (PBR) and optimum sustainable population (OSP) determinations for all species under the MMPA, as well as to implement recommendations in the Draft Steller Sea Lion Recovery Plan and provide

information upon which to evaluate delisting criteria. As such, the proposed activities would contribute to the conservation and management of, and acquisition of necessary biological information for, Steller sea lions in the southern extent of the eastern DPS range.

Our specific objectives include:

(1) Assess status and trend in Steller sea lion population size.—Recovery criterion #1 (NMFS 2006) for delisting of the eastern DPS of Steller sea lions is an increase in the population of at least 3% per year for 30 years. Our objective is to provide information from the southern extent of the eastern DPS in order to help evaluate whether this criteria is being met. This activity is a continuation of over 20 years of aerial surveys that we have conducted in Oregon and northern California (see Fishery Bulletin 107:102-115). In addition, survey results provide the necessary baseline population data required for estimating Potential Biological Removals under the MMPA.

H1: The Steller sea lion population in Oregon (a component of the eastern DPS) is increasing at 3% or more per year.

(2) Assess status and trend in Steller sea lion population ecology and vital rates.— Recovery criterion #2 (NMFS 2006) for delisting of the eastern DPS of Steller sea lion is a finding that the "population ecology and vital rates are consistent with the trend observed under criterion #1, to ensure the population is increasing in a sustainable manner. Specifically, available information on pup counts, fecundity, juvenile survival rates, population age structure, gender ratios, and other observations should be examined to determine that they indicate an increasing population." Our objective is to provide information from the southern extent of the eastern DPS in order to help evaluate whether this criteria is being met. This activity is a continuation of five years of research on juvenile survival rates, and over a decade on food habits, pups counts, and other observations.

H2: Steller sea lion vital rate parameters in Oregon (a component of the eastern DPS) are consistent with an increasing population.

(3) Compare and contrast results from (1) and (2) with concurrent studies in the western DPS and in the northern extent of the eastern DPS range.—Our research is coordinated with, and complimentary to, that being done by NMFS and the Alaska Department of Fish and Game (ADFG) in the western DPS and in the northern extent of the eastern DPS range. Our objective is to compare and contrast estimates of vital rates and associated parameters in order to better understand the causes of why some Steller sea populations are increasing while others are decreasing; and in so doing, aid the recovery of those populations in decline.

H3: There are no significant differences between vital rates parameters measured in the eastern and western DPS segments (of which the Oregon population is a component).

15. The applicant is quite specific in requesting the capture and sampling of 10 adults and 200 pups. The application does not specify why all adults are subjected to all procedures but not all pups are (e.g., 25% of pups receive fecal loops and culture swabs, 80% of pups will have scientific instruments attached). Nor does the applicant explain the origin of sample sizes requested other than to state generally that "sample size is sufficient for drawing reasonable inference." How he has arrived at this conclusion is unclear.

Statistical power analyses were conducted to determine the total number of pups that would be needed each year to estimate juvenile survival rates. Two hundred animals (100 each sex) was necessary to detect a 30-60% decline in survival from 0-4 years with 80% power assuming a detectability of 30-50%. Power analyses were also conducted to determine that 80 branded pups would need to be equipped with scientific instruments to evaluate the affects of branding on survival and emigration of pups from the rookery (80% power to detect at least 10% change with 95% confidence). It is not necessary to sample the entire population with fecal loops to establish the prevalence of parasites; a sample of 50 animals is generally suitable.

16. Although the applicant states on page 20 that there are no known alternatives to the research proposed, and states that the "tools and methods proposed in this application are state of the art." this is apparently not true. As noted above, aerial photogrammetry has been shown in literature (and substantiated by the DEIS) to be sufficient for accurate population census. It is proposed for use by other permit applicants, and has been used for years to census pinnipeds in most other parts of the country, yet this applicant continues to propose the use of drive counts, arguably the most intrusive manner of counting animals. This activity should not be permitted.

See first paragraph of response to comment #13.

17. There are also some inconsistencies in statements. For example, page 14 states that culture swabs will be taken from "as many as 50 pups annually *and any other handled sea lions* with lesions…" [emphasis added] In fact, the applicant requests swabs for *only* 7 50 of the 200 captured pups and for all 10 captured adults. Thus he cannot take swabs from "any other" animals regardless of whether they are showing signs of lesions. Further, page 18 states that skin biopsy, fecal loop and culture swab sampling will be collected while animals are under anesthesia, yet the applicant has previously stated that adult Steller sea lions will not receive anesthesia during branding (see page 15).

We withdraw the language on page 14 "…*and any other handled sea lions.…*" We request the language on page 17 (not page 18 as reviewer indicated) "…*while the animals are…*" be replaced with "…*while pups are…*".

18. We also wish to raise the issue that adults will not receive anesthesia for branding. The rationale for denying anesthesia to adults under this permit is not made clear

other than the vague statement that they can be "restrained more efficiently and safely using the squeeze cage." Other permit applicants [e.g. Horning and Trites] have discussed the pain involved and have stipulated that they would provide anesthesia during branding and other potentially painful procedures; it is not clear why this applicant would not. Denying anesthesia for painful procedures clearly violates the MMPA's requirement that research be humane, particularly in light of admissions in the DEIS that burns from branding result in the formation of blisters…and fluid seeping from the burned area and are accompanied by severe pain." [DEIS at B-22]16 U.S.C. § 1374(b)(2)(B); 50 C.F.R. § 216.34(a)(1). NMFS may not grant a permit unless the research practice causes the "least possible degree of pain and suffering practicable." 16 U.S.C. § 1362(4).

We no longer plan to study adult Steller sea lions and therefore withdraw all requests related to their take.

> In addition, of the total of 240 animals to be captured, branded and sampled each year under this proposed permit, permission is requested for 10 incidental mortalities. This equates to a mortality rate of approximately 4%. This rate is substantially higher than that projected in the DEIS for these types of activities (see tables in DEIS at 4-53 and 4-54). If the applicant believes that there are likely to be fewer deaths, fewer should be requested.

Only 210 animals (200 pups, 10 adults) were requested to be handled , not 240; the current request is to capture and brand 200 pups annually. We request that are incidental mortality take be reduced from 10 to 5.

19. Although the applicant attempts to quantify the mortality risk from branding on pages 16-18 of the application, the estimates not only do not comport with rates of risk in the DEIS, they do not even reflect deaths that have occurred in Oregon. The applicant fails to cite work by Scordino (2006) that documented pup mortalities that occurred in single year in Oregon at the Rogue Reef Refuge in the wake of branding and sampling activities. We also note the caveats in the DEIS that post-monitoring work is rarely done and that even animals that appear to be calm during handling can suffer post-capture myopathy. This fact, and work by Scordino, argue for the need for careful, systematic post-capture monitoring to occur.

See response to comment #6, #9, #10, and #11. Scordino (2006) is in reference to a graduate student's Master's thesis and is not a peer-reviewed scientific paper. Data collected concurrent and subsequent to the Scordino thesis project are still being analyzed. The complete analysis of all data related to this study will likely demonstrate higher survival rates for branded pups than what was supported by the smaller dataset of Scordino.

20. We believe that this application provides insufficient and inconsistent information regarding likely impacts. It is not clear that the applicant can meet the test

required by the MMPA that the methods used are those less likely to cause pain and suffering and are the most risk averse of available technologies.

We disagree. See above for response to reviewer's specific comments.

Annual and Final Reports

Please be advised that all reporting requirements for your previous vacated MMPA and ESA permits, including annual, final, and special reports required under specific permits still apply. Therefore, all research that was conducted and not included in your 2005 annual report must be submitted before new a new permit can be processed. Even if no work was done, annual reports are still required stating such, as are final reports for any permits that expired or were vacated.

Comments received on File No. 1034-1887 (Markus Horning)

Marine Mammal Commission (MMC)

1. Additional information is needed about how and how long animals implanted with Life History Transmitters will be monitored for injuries or death related to implantation procedures.

Implanted animals will be monitored in three different ways for injuries or death related to implantation procedures:

a) *All LHX animals will be monitored after release using external satellite-linked data recorders (SDRs). SDRs provide data on survival and behavior (see Thomton et al., in review, a,b):*

Thomton J, Mellish J, Horning M. (in review, a) Effects of temporary captivity on diving and ranging behavior of juvenile Steller sea lions, Eumetopias jubatus. Marine Ecology Progress Series

Thomton J, Mellish JE, Horning M. (in review, b). Dive behavior and movement patterns of juvenile Steller sea lions after intra-abdominal transmitter implantation. Submitted to Journal of Experimental Marine Biology and Ecology.

This technique is limited through constraints on attachment duration. Depending on time of year at release, and how close animals are to moult, SDRs permit monitoring for about 2-16 weeks. The six juvenile Stellers released to date were successfully monitored for a little over 2 months (minimum) to 4.5 months (maximum).

See also response by the ASLC to comment #46 in permit application 881-1890, quoted here (with permission):

"An in-depth study of the post-release behavior of all juveniles through the transient program to date (including LHX implant recipients) is currently in review (Thomton et al., in review a,b). A brief discussion of the relevant findings for all animals, post-release is included below. However, it should be noted that all animals, including those with dietary challenges, must be within acceptable mass (e.g., within 15% of entry mass) and body fat (10-25% total body fat) ranges prior to release.

Juvenile Steller sea lions released from the temporarily captive facility were tracked with externally attached satellite-linked data recorders for 74.1 ± 9.6 days (range 14 - 160 days). The mean dive depth (26.2 ± 4.0 m), dive duration (1.4 ± 0.1 min), dive rate (10.1 ± 0.5 dives hr-1), trip duration (14.1 ± 2.1 hr), haul-out duration (11.2 ± 1.5 hr) and time wet (46.9 ± 2.6%) were within the range of previously published values. Movement (190.0 ± 31.9 km) between haul-outs and rookeries during the tracking period was also typical of juvenile Steller sea lions in Alaska. Following release, juvenile Steller sea lions performed

shorter and shallower dives during the first week post-release, than free-ranging conspecifics. However, all parameters were within expected ranges for juveniles of the same population by the second week post-release. The overall mean dive depth, dive duration, trip duration, haul-out length, dive rate, percentage of time wet, and dispersal were equal to or greater than the FR control and published juvenile SSL values. Furthermore, during the 2.7 mos tracking period, TJs appear to equal adult female diving abilities. The results of this study indicate that temporary captivity of up to three months probably does not impair dive performance or dispersal. In addition, dive development may continue until at least 24 mos of age.
LHX implant recipients - During the 91.5 ± 8.6 day tracking period following release from temporary captivity, all sea lions returned to their respective capture haul-outs. Dive depth, duration, frequency and dispersal distances were similar to non-implanted animals. A possible captive recovery effect was observed during the first week post-release when dive depths were shallower and durations shorter than free-ranging juveniles. By the second week all dive behavior parameters were within the normal range and did not differ from non-implanted animals. This effect was also observed in non-implanted individuals maintained in temporary captivity. In conclusion, surgical implantation of LHX tags does not appear to have a negative effect on the short-term dive behavior or movement of juvenile sea lions."

b) *Animals will be monitored through opportunistic video-based (e.g. Chiswell remote video system of the ASLC) and resight trip-based observations. Two of four LHX animals relased in April 2006 were subsequently resighted via the video monitoring system. Of three juveniles release in November 2005 (two of these had LHX tags), one was subsequently resighted.*

Successful resighting through video or direct observations requires branding and brand recognition for positive identification. In all instances, flipper tags would have been too small to allow positive identification of animals. See also response c) to comment #3 (by USDA) below. See also response to comment #21 in application 782-1889 (NMML) for details on resight efforts, quoted here with permission:

"NMML collects observations and photographs of marked animals each year both from land-based and skiff-based observers. Land-based observations primarily come from two islands with rookeries where pups have been branded every other year since 2000: Marmot and Ugamak Islands. Researchers are stationed on these islands at field camps for 2+ months from late May through early August each year and collect a wide variety of information of sea lion attendance, behavior, and observations of marked animals from cliff-side observations posts high above the rookeries. Land-based observations from field camp-based researchers are conducted each day with no disturbance to the animals, since they are unaware of the presence of researchers."

"NMML also conducts brand-sighting cruises during which researchers observe sea lions from small skiffs just offshore of haulouts and rookeries in the Gulf of Alaska and Aleutian Islands. During these cruises, researchers may also go ashore in some locations to observe sea lions from vantage points that are preferable to those available from the skiff. Sea lions are aware of the presence of researchers during skiff-based observations, and most will become alert, look at the researchers and vocalize. Researchers try to avoid large disturbances, such as those that might cause them to enter the water, since this would preclude further effort to record sightings and photograph marked animals. Researchers may (weather and time permitting) visit sites with large numbers of sea lions on multiple occasions, sometimes on successive days. This is because groups of sea lions that use a particular site may be at-sea during the first day of observation and haul-out on the second."

Other parties conduct resight efforts (e.g. ADF&G), but these data are collated by NMML, from whom we will receive information on resights of LHX animals:

"NMML also receives information about sightings of Steller sea lions marked by NMML from other research organizations (e.g., Alaska SeaLife Center, Alaska Department of Fish and Game), as well as native Alaskan organizations (e.g., The Alaska Sea Otter and Steller Sea Lion Commission) and members of the public."

And from responses to comment #4 on application #358-1888 (ADF&G, with permission):
"ADFG is committed to resighting surveys throughout Southeast Alaska every summer for the next 10 yrs, given funding is available for this priority work."

c) *Animals will also be monitored through the LHX tags. If data returns from LHX tags indicate external traumatic death (e.g. death by predation by orcas), this simultaneously confirms absence of death or significant injury through the tags until the moment of predation.*
See also our response below, to comment #18 by the HSUS, about recent data returns from LHX tags.

We would like to point out that LHX tags are currently the only way to positively monitor individual animals for their entire life, and to obtain information on their fate.

2. In describing the platform method of capture, the application does not, but should, state the maximum time that animals would be held in the holding cage until they are sampled, released, etc.

A group of up to 10 adult sea lions can be handled, marked and released in roughly two hours or less. Animals are continuously monitored during the entire process.
From responses provided by the NMML to comment #6 on permit application 782-1889 (quoted with permission):
"Based on floating pen captures at Shilshole Marina in Washington, sea lions are rarely held for longer than 2 hours. The ultimate holding time will depend on a combination of number captured and procedures performed. Capture pens target large juveniles and adults and are open to the air. In the unlikely event of potential overheating animals can be sprayed with cold ocean water."

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3. While there may be reasons to use hot branding for permanent identification, the activities in the multiple Stellar [sic] sea lion and northern fur seal applications do not warrant its use. The study design does not require and does not benefit from capture/release/recapture. All biochemical and body morphology do not require multi-year sampling of identified individuals. Any identification needed in one year to prevent resampling of the same animals can be served well by bleach or paint.

   Branding should not be used unless it is the only means of identification possible. Since the protocols do not call for monitoring specific individuals as a key component, hot branding is not acceptable for these permits.

PR1: Please explain why your study cannot be accomplished without hot brands.

There are multiple reasons for branding:

a) *Branding LHX animals will be very important for the long-term, opportunistic monitoring of these animals through existing video-based and trip based observations – see reply to comments by the Marine Mammal Commission above. Ultimately, comparing resight rates between LHX and non-LHX animals will provide the best assessment of any long-term effects of LHX tags or procedures, one of the goals as specifically stated in the application.*

b) *It is important to avoid re-capturing LHX animals for other projects (this consideration actually applies to all projects except for those that specifically use recaptures as part of their experimental design). This is important beyond the first year after release. In particular for underwater captures, brands are the only long-term, reliable positive identification recognizable underwater.*

c) *Conversely, a combination of LHX and branding approaches may be the most effective way of assessing the long-term impact of branding on survival, if, as is being considered, only a subset of LHX animals will be branded.*

(See also response to comment #1 by the Marine Mammal Commissions).

d) *Every single scientific peer or panel review conducted over the past 10 years on Steller sea lion research has consistently highlighted the need for multiple studies based on comprehensive, positive, long-lasting individual identification, and specifically branding-based demographic studies. Most reviews and comments received have also underlined the need to continue the ongoing efforts to coordinate multiple research projects. By distributing branding across all projects that manipulate animals in a manner that allows branding, the overall number of animals that need to be captured and manipulated for multiple separate projects can be minimized.*
Thus, in this case branding LHX animals (and any animal that is manipulated in a manner to permit branding), is in fact directly addressing the REDUCTION components of the three R's (see USDA comments below).

Please also note responses given to comment #8 on permit application 782-1889 by the NMML, which refers to their previously listed justification for branding, and which also provides additional verbiage in relation to this justification. Since our branding efforts are coordinated (see also point d above), we are using the justification for branding provided in application 782-1889 (quoted here with permission):
"…we included a thorough justification of this method on pages 54-76 of our application as "Appendix 1. Justification for and Summary of Hot-iron Branding on Steller Sea Lions Including Branding on Other Pinnipeds." This text was also included in the Alaska Department of Fish and Game application, but presents justification and analysis of this technique applicable to NMML, ADFG, Oregon Department of Fish and Wildlife, Alaska Sea Life Center, and Oregon State University permit applications for Steller sea lion branding."

From response to comment #6 on application #358-1888 (ADF&G, with permission, and this text is also used in applications by other applicants):

"To summarize, many different methods of marking pinnipeds have been developed and tested. Hot branding is currently the only known way to permanently mark pinnipeds with codes that can be easily read from a distance, year after year, throughout the animal's life. In coordination with NMFS National Marine Mammal Laboratory, we have chosen letter designations for our study rookeries (as is done elsewhere throughout the range of Steller sea lions in US and Russian Waters). Each individual pup handled at a rookery is marked with that designator followed by an individual number. Each individual pup handled at a rookery is marked with that designator followed by an individual number (1, 2, 3, …, 43,…..211,……etc.).

The placement of such a large amount of hot iron to the skin of the animal would concentrate far too much heat in the iron cluster, resulting in an inconsistent transmission of heat and greatly increase the risk of over-branding. Even if the reviewer had suggested a new method of hot branding that avoided this increased risk of over branding, it would not be possible to apply more than one mark (number/letter) at a time. Each digit is carefully applied on a specific spot with extreme care and attention to

pressure and time of application. Since the animal's body is not flat, a device designed to place all marks at once would remove the ability to monitor each mark and would result in unacceptable variability in the quality of the mark. Finally the application of all digits at one time actually works against one of the methods that we have been trying to use to resolve unreadable brands. We have been purposely "randomly" applying one of the digits off of a straight horizontal line (either high or low) so that it gives us one additional
characteristic in the brand photo to compare against future sightings in the event that one of the digits grows/heals to be unreadable. A fixed branding iron would eliminate this additional criteria used to distinguish individuals, thereby reducing resighting ability and ultimately result in a reduced ability to meet the stated objectives."

4. In addition, under the AWA, any time a potentially painful or distressful procedure is to be used, alternatives must be researched and final choices must be justified for the study. In this consideration of alternatives, reduction of numbers, refinement of technique, and replacement with other animals must be considered. If a study exists that requires hot branding (not considered humane), the length of time the branding takes, post procedure treatment and alleviation of pain, and other options must be considered. In the studies under review, there is no justification for using 4 digits, or for branding each digit alone.

   At a minimum, 3 digit branding is sufficient to mark more animals that needed for the studies (34X34X34 – over 39,000 combinations, using only 0 and 1, not "o" and "I"). Additionally, devices should be used that load all three irons together so that only one application of the brand is used (20 sec as opposed to 2 minutes).

PR1: Please explain the need for 4 digit brands, i.e., why aren't 3 digit brands used. Please also explain why each digit is applied separately.

We are applying the standard branding protocol developed by other projects, for consistency purposes. The same justifications used in the applications describing the standard branding protocol is applicable to branding within our project, and is added verbatim (with permission) from the NMML responses to the comments received on permit application #782-1889 (comment #9):

"The comment reiterates evaluation criteria that must be considered when choosing and justifying the application of potentially painful or distressful procedure on study animals, all of which were carefully considered in the design of the Steller sea lion survival and vital rate study program. Descriptions of the branding method, including the choices of branding digits, possible effects on the animal, and methods employed to reduce stress, pain and suffering were included in Appendix 1 of our permit application, "Justification for and Summary of Hot-iron Branding on Steller Sea Lions Including Branding on Other Pinnipeds." This text is also applicable to hot-branding proposed in the Oregon Department of Fish and Wildlife, Alaska Sea Life Center, and Oregon State University permit applications for Steller sea lion studies.

To summarize, many different methods of marking pinnipeds have been developed and tested. Hot branding is currently the only known way to permanently mark pinnipeds with codes that can be easily read from a distance, year after year, throughout the animal's life. In coordination with other researchers in the U.S. and Russia, letter designations leading a brand digit sequence specifically designate the natal rookery if branded as a pup. Each individual pup handled at a rookery is marked with that designator followed by an individual sequential number. Choice of digits and combinations included considerations of potential for observer error in recording subsequent observations of a marked sea lion.

If all digits were combined on a single iron, the placement of such a large amount of hot iron to the skin of the animal would concentrate far too much heat in the iron cluster, resulting in an inconsistent transmission of heat and greatly increase the risk of over-branding. Even if the reviewer had suggested a new method of hot branding that avoided this increased risk of over branding, it would not be possible to apply more than one mark (number/letter) at a time. Each digit is carefully applied on a specific spot with extreme care and attention to pressure and time of application. Since the animal's body is not flat, a device designed to place all marks at once would remove the ability to monitor each mark and would result in unacceptable variability in the quality of the mark, and increase a risk of injury to the sea lion. Finally the application of all digits at one time actually works against one of the methods that some researchers have implemented to resolve unreadable brands, purposely "randomly" applying one of the digits off of a straight-horizontal line (either high or low) so that it provides one additional characteristic in the brand photo to compare against future sightings in the event that one of the digits grows/heals to be unreadable. A fixed branding iron would eliminate this additional criteria used to distinguish individuals, thereby reducing resighting ability and ultimately result in a reduced ability to meet the stated objectives."

5. No consideration has been made for post procedure treatment with antibiotics or pain relief has been addressed. All facilities would be required to have an approved protocol from their IACUC that has shown consideration of alternatives and use of methods that would alleviate discomfort, stress, and long-term complications. Topical antibiotic/anesthetic cream should be used post procedure.

PR1: Please explain what considerations have been made for post-procedure analgesia and antibiotics.

We are applying the standard branding protocol developed by other projects, for consistency purposes. Very much consideration has been given to the issue raised by the reviewers in comment #5 above. From the responses to comment #10 in application 782-1889 (NMML): (with permission):

"Contrary to the reviewer's comment, a great deal of consideration has been made for

post-procedure treatment of Steller sea lions. The narrative below addresses the comments specifically. The NMFS does not have an IACUC for research covered under the MMPA and ESA. However, due to the urging of the Marine Mammal Commission, the NMFS is currently considering developing policy which might require IACUC review for all but observational studies covered by MMPA and ESA Research Permits.

However, this does not mean that the methods used for hot branding have not gone through any IACUC review. Each of the groups requesting branding of Steller sea lions in their permit applications coordinate to follow the same protocols, use similar if not the same equipment and often share personnel and veterinary services. These protocols have been reviewed by the ADF&G Division of Wildlife Conservation IACUC committee and the University of Alaska, Alaska Sea Life Center IACUC committee and these responses come from a coordinated effort between the groups.

The ADF&G Division of Wildlife Conservation IACUC has reviewed and approved the protocols for animal use for this permit application. Alternatives and methods for relieving stress and discomfort were addressed as required. All painful procedures are conducted under general anesthesia. The use of topical anesthetic cream as a post-branding analgesic does not show any promise. Topical anesthetic creams only penetrate 2mm on mucus membranes, are not recommended for wounds, and have a short duration of effect. Topical anesthetic cream are recommended for use on intact skin for procedures such as catheter placement or blood collection. However, the cream must be covered by an occlusive dressing, takes at least 20 to 30 minutes to reach anesthetic effect, the peak of which is at 2 hours (Mathews 2005). In addition, they are generally water soluble, impair healing and would not have any prolonged analgesic effect once the animal entered the water. Thus, they are not indicated for the types of wounds created in the described procedures. Topical antibiotic ointments are <u>contraindicated</u> for deep puncture wounds (as listed on the label) and thus should not be introduced in to biopsy wounds. Topical antibiotic creams include Nolvasan® (which is water soluble and washes off immediately with water) or Silvadene®. Silvadene® is used against bacteria or fungi for human burn patients and could be applied post branding but is normally applied 1 to 2 times daily and kept covered until the wound heals. Thus, applying once, immediately post branding and prior to the port-branding tissue sloughing would probably have a negligible effect and would of course require re-capture of the animal for application thus making it's use a requirement of additional disturbance.. We do not use antibiotic ointments topical anesthetics in the biopsy wounds as this is contraindicated, contrary to the tenets of wound treatment in veterinary medicine, and increases the likelihood of an anaerobic infection and.

Injectable analgesics have been considered but the risks and short duration of action outweighed the brief post-procedure benefits. Burtorphanol, a narcotic agonist/antagonist could be administered at a dosage used for domestic carnivores of 0.5 to 0.4 mg/kg subcutaneously. This would likely give some analgesia for 6 to 8 hours. However, it does have the risk of CNS or respiratory depression, either of

which we want to avoid in a free-ranging, diving mammal and therefore the risk outweigh the benefits. Buprenorphene is another narcotic and has a longer duration of action, 8 to 12 hours. However it is a controlled narcotic (30 times more potent than morphine) and has a higher risk of CNS and respiratory depression than butorphanol and poses an enormous risk for the personnel handling the drug. The non-steroidal anti-inflammatory flunixin meglumine (Banamine®) has been used by marine mammal veterinarians for post-surgical wound pain. It is given at a rate of 1.1 mg/kg IV or IM. The duration of action in pinnipeds is unknown but in carnivores it is given once per day. This is a drug used most often in horses and ruminants, though dogs are highly sensitive to adverse effects on the gastrointestinal lining (such as gastric ulcers) and thus is not approved for use in dogs. A single dose in a pinniped is probably safe but there isn't any published information on the safety or efficacy in these species and therefore its use in this situation is not supported. Meloxicam is another NSAID considered but is it excreted at higher concentrations in milk than in plasma in rats so would not be a good choice for the lactating female. There is no recommended dosage for marine mammals so metabolic scaling would need to be employed to estimate a dose. In reference to the hot-branding of pups, the NSAIDs are not recommended for animals less than 6 weeks of age based on the development of the liver and kidney and should therefore be avoided in rookery aged pups (Mathews 2005). In comparison to the alternatives (i.e. narcotics with respiratory depressive effects), fluninxin meglumine is the best choice we have for juvenile and adults but still carries risks. There isn't a readily apparent safe alternative injectable analgesic for pups on the rookery indicated for extended analgesia post-branding.

Local anesthesia was considered for analgesia post-biopsy and branding. However, a local infusion could be used and for branding would require such a large area would need to be infused and large volume of lidocaine or buprivacaine that the dose could be toxic. If used it would need to be diluted in saline, buffered with sodium bicarbonate and warmed to body temperature. Additionally, infiltration of lidocaine is extremely painful in the human neonate or pediatric patient (Rodriguez and Jordan 2002) and must be administered very slowly. Animals are likely to have a similar hypersensitivity because of their immature peripheral nerves. An inverted L block could be possible but even for the rookery pup, animals would still need to be anesthetized first (because of the extreme pain of the injection) and held much longer under anesthesia for the prolonged injection time and to wait for minimum 10 minutes for the block to take effect putting the rookery pup at higher risk from prolonged gas anesthesia on a mask without intubation. A spinal block would paralyze the swimming ability and a regional nerve block procedure for pinnipeds is unknown (we don't have the landmarks blocked off."

Mathews K A. 2005. Analgesia for the pregnant, lactating and neonatal to pediatric cat and dog. J Vet Emergency Critical Care. 15(4): 273-284.

Rodriguez E and Jordan R. 2002. Contemporary trends in pediatric sedation and analgesia. Pediatric emergency medicine: current concepts and controversies. Emergency Med Clin North Am. 1:199-222."

6. No consideration was given to proper recovery times for anesthesia. While an animal may be awake and mobile within 20 minutes of cessation of isoflurane, it is a recognized occurrence in veterinary medicine that the effects of anesthesia do not dissipate after 20 minutes. In practice, we have seen animals take up to 24-48 hours to recover from anesthesia, especially when the procedure of lengthy (over an hour).

PR1: Please explain what considerations are made for recovery of animals from anesthesia versus the initial return to consciousness.

There is no mention of a 20 minute recovery period in our application. Recovery periods are subject to substantial individual variation, and thus no set recovery periods should be used. Instead, attending personnel should monitor animals and adjust recovery periods as needed, ad hoc.
Our application states that following LHX implant procedures, animals will be monitored for a minimum of 24 hrs. Animals will only be given access to water once attending veterinarian(s) and P.I. or co-I. deem such action to be safe.

From response to comment #18 to application 881-1890 (ASLC, with permission), a description on the recovery process and the experiences this is based on:

"Animals are closely monitored throughout the anesthetic recovery process from the initial return to consciousness through the ability to move and react normally. In 10 years of experience with over 400 isoflurane procedures on captive Steller sea lions (most of which lasted greater than 2 hours) we have consistently observed recovery to full function in 30 to 60 minutes as long as no other sedative medications are used (P. Tuomi, DVM, pers com). Due to the logistical advantages of the quarantine facility, animals are allowed to recover in a dry location. Access to water is not granted until the Chief Scientist and Attending Veterinarian deem it safe based on the stable movement and normal behaviors (i.e., grooming, response to visual stimulation, interest in food, etc.) of the individual. During field capture situations, animals are allowed to recovery in the safety of the capture or transport box. When the animal is deemed safe to release, the door is opened such that the animal can leave of its own will when ready."

Thus, all our procedures are designed in accordance to recommendations issued by multiple veterinarians that routinely conduct associated procedures (anesthesia, surgery etc…) on pinnipeds, and is thus based on the most expert opinions available in the field, and on experience of several hundred such procedures under actual, realistic conditions, on target and related species. These procedures are also subject to stringent reviews by pertinent IACUCs.

7. While small surface skin biopsies may be acceptable without anesthesia under some conditions, proposals for blubber and muscle biopsies, some up to 2 ½ inches deep, constitute painful and invasive procedures and must be done under anesthesia. If local anesthesia is to be used, dosages should be given, and well as documented protocols for determination of effectiveness, including the waiting period for full effect.

PR1: Please provide details about use of anesthesia for blubber and muscle biopsy, including dosages, how you determined the specific anesthetic/dosage would provide appropriate level of anesthesia, and how long you would wait post-deliver for the anesthetic to take effect. If you do not intend to use anesthesia for these procedures, please explain why.

Our application does not request any muscle biopsies to be taken. Our application does not request blubber biopsies to be taken without anesthesia. All blubber biopsies will be taken only under anesthesia.

8. The amount of blood expected to be taken at sampling, while below 10% of blood volume, are significantly high. Given the state of current laboratory methods, it seems that samples can be much smaller, as most tests no longer require 5 ml of serum anymore, more like 0.1 ml. Remember refinement and reduction – this can be applied to sampling as well.

PR1: Please explain why your specific studies require collection of the amount of blood requested in your application.

This comment suggests unfamiliarity with pinniped blood volumes, requested sampling volumes, and with volumes required for laboratory analyses. Our application does not request to take up to 10% of blood volume. Our application specifically states a maximum of ½ of that amount, and in all situations where permissinble, even substantially less. Quantities will be collected as needed for modern assays, and thus reduction and refinement will be implemented whenever possible. Some assays require "more like 0.1 mL serum" is not an accurate, well-informed statement: some of our samples are assayed by commercial laboratories, and these stipulate specific quantities. For example, commercial laboratories that analyze liquids for body composition, or e.g. blood volume using tracer dilution, request a volume of 1 mL for each sample (e.g. Metabolic Solutions). Multiple samples are required for background and establishment of equilibration. In addition, a given volume of serum requires a larger volume of whole blood, typically for diving animals with high hematocrits, serum extraction ranges from a low of 20% to a high of 50% of blood sample. Thus, using the example of a tracer dilution requiring 1 mL of serum for sample analysis, a single pre-injection background sample is required,

plus up to five post-injection samples to determine equilibration curves (depending on tracer), for 6 mL of serum, or using a serum extraction efficiency of 25%, 24 mL of whole blood are required (just to give one example). Although the volumes for individual analyses may be very small, there must also be consideration for the repetition of analysis within each assay for QA/QC. All Steller sea lion researchers are also very committed to trying to archive serum in cooperative ways, for future research to provide baseline data for retrospective analyses.
In summary, we only sample the required volumes for the specific assays and archival purposes listed in the permit application, based on the most recent tests and stated volumes for such tests. All procedures pass stringent reviews by all applicable IACUCs in terms of processes and collected volumes. The proposed quantities meet accepted laboratory animal husbandry practices.

9. As discussed above, all should address the potentially painful procedures and care of the animals.

PR1: Please explain measures that would be taken during and after potentially painful procedures.

This is already explained in detail in the application, in section D. Effects and Mitigation Measures, subsection 2. Measures to minimize effects. Maybe the confusion stems from the fact that in previous permit applications, mitigation measures were listed directly with the specific treatments, whereas in this application the specified format required listing mitigation in a separate section. Virtually all procedures listed in this application will be conducted under gas anesthesia. All treatments that require mitigation have appropriate mitigation measures listed. Additional measures are listed under 'contingency measures'.
In addition, all procedures listed in this application are subject to rigorous internal reviews by all applicable IACUCs, including scrutiny for proper mitigation measures where pain may be involved. Copies of these AUPs have been / will be provided to OPR.

10. Applicants identify that studies have already proven that certain methods are equivalent to the "gold standard" –deuterium(?) measures – for determining body composition. Therefore, it appears to be redundant and unnecessary for more than one method be used, increasing the handling and sedation/anesthesia. Only one method for determination of body composition should be used for each study. The intent of the studies is to monitor the animals, not compare methodologies. As an endangered species, these sea lions should be studied, not experimented on.

PR1: Please explain why it is necessary to use multiple methods on the same animal for determining body composition and why comparison of the methods cannot be performed using a non-ESA listed species.

Our application does not include multiple methods to determine body composition.

The only requested method specific to determining body composition, is labeled water dilution (deuterium oxide, or heavy water). This method delivers an estimate of lean tissue mass, which in connection with body mass measurement allows the estimation of body condition, if that is defined as percent adipose tissue.
The only other method we will apply that could be confused with body composition methods, is maybe ultrasound based measurements of blubber thickness. This measure is not redundant with isotope dilution. Isotope dilution is 'as mentioned above' the 'gold standard' for % adiposity or body composition. However, this method cannot distinguish between subcutaneous and other body fat stores (e.g. intraperitoneal, although admittedly pinnipeds have little of the latter), and it can also not deliver data on regional differences in fat stores, and their depositions and mobilizations (see Mellish, York & Horning 2007). These data on regional differences however are very important for our efforts of developing models to estimate body condition trends through remote morphometric, photogrammetric, and infrared based thermal pattern measurements.

> *Mellish JE, Horning M, York AE. 2007. Seasonal and spatial blubber-depth changes in captive harbor seals (Phoca vitulina) and Steller's sea lions (Eumetopias jubatus). Journal of Mammalogy 88 (2): 408-414.*

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11. Page 15, choice of species: while poor juvenile survival was certainly a proximate factor during the large decline of western stock Steller sea lions in the 1980s, declines since the 1990s were associated with low fecundity (Holmes and York 2003). The justification for studying juveniles in this paragraph should be rewritten to address conclusions of Holmes and York (2003).

The NMFS comment is not quite accurate: it has been suggested that poor juvenile survival was a proximate factor of the decline in the 80's, but this hypothesis has not been tested to date. Similarly, Holmes and York advanced the suggestion that low female fecundity may explain more recent population trends, but once again, this was shown within the context of their modeling efforts, this is not a tested hypothesis. We have clearly stated that in the relevant sections of background, objectives, justifications etc.

12. Does this study also depend on LHX tag deployments proposed under application 881-1890 by the ASLC?

No, this study does not depend on deployments under the ASLC application.
The deployments under the ASLC application – if conducted – would replace an equal number of deployments under this application. This will be coordinated with the ASLC, and Dr. Horning is listed as a co-Investigator of the ASLC application (#881-1890), and Dr. Mellish (ASLC), Dr. Gelatt (NMML), and Dr. Rea (ADF&G) are listed as co-I's on this application.

The Humane Society of the United States (HSUS)

13. Some of the citations in the text appear to be missing from the bibliography. We did not have time or intent to search the document, but two citations in which we

were interested were not listed (e.g., Link and Barker, 2005; Mulcahy & Garner, 1999). The applicant should check to be sure that the bibliography is complete.

Done, updated bibliography is attached.

14. The applicant has requested the use of some procedures that were not discussed and whose effects and mitigation were not mentioned in the DEIS (e.g. use of deuterium dilution and some aspects of stable isotope analysis) and thus these procedures should not be used.

Stable isotope analysis is covered under the specific sample collection method requested, under section 2.19 of Appendix B of the PEIS. Deuterium dilution is covered under section 2.6 of Appendix B, administration of drugs, and section 2.11, venipuncture and blood collection. Deuterium oxide is simply heavy water, and has no known side effects. Therefore, the risk assessment associated with Deuterium oxide injection and subsequent blood sampling are covered under drug injection and blood sampling, respectively. These procedures are included in the PEIS, and their effects and mitigation are described.

15. A major portion of this application is seeking permission to surgically implant two life history (LHX) transmitters in up to 100 Steller sea lions from the endangered Western stock over a period of five years. There is some confusion about sample size that requires urgent attention. The applicant's text summary on page one states that he seeks permission to implant devices in 20-50 Steller sea lions per year for a total of 100 animals. Yet the chart (table 1a) indicates that this is 100 animals *per year*, for an apparent total of *500 animals*. This discrepancy must be addressed. For purposes of these comments, we will assume that it is 100 animals over a 5 year period, as this number is also used elsewhere in the text.

As the chart and text both state, and as repeated here for clarity, the maximum annual take or maximum annual sample size (thus, permitted annual maximum – as in how many animals we can at the very most do in one year, this is the 4th column in Table 1) is 140 captures, including 100 implants. It is also stated that the total take for the entire project – as in how many animals can we maximally work on in all years of the project combined (this is the 5th column in Table 1), is also 140 captures, including 100 implants. Thus, the maximum annual and total project takes are identical. How can that be? This simply means that we may work with all animals required for the project in one year (though this is unlikely), or we may work with fewer than the permitted annual / total, in each of multiple years. The text states that the project will most likely (but not necessarily) be spread over 2-3 years. Table 1 merely states the MAXIMUM, not the ACTUAL. Under no circumstances can we or would be work with more than the clearly stated permitted total take for the entire project.

16. The applicant would work in conjunction with the ASLC, which is also seeking to implant up to 30 LHX, that may comprise a subset of this applicant's desired sample size of at least 92 animals per year. Further, the application by the ASLC seeks to attach a multiplicity of devices to captured animals. We believe that the

physiological stress of surgical procedures required for the LHX, and its attendant risk should preclude the attachment of other devices (e.g., buoyancy "challenges" and heart rate monitors) which may themselves add to the animal's burden and compromise reliability of data gained from the LHX. To adequately control sampling, standardize protocol, and minimize risk to animals, we would prefer to see that only one permit be granted to explore this technology, if NMFS grants a permit for its use.

Any animals receiving LHX tags under the ASLC's permit, will be used to fill the sample size requested here, and will count towards that. Thus the deployments under the ASLC application – if conducted – would replace an equal number of deployments under this application.

Our recent studies show no evidence that the surgical implantation procedures, recovery periods, or having implants, are a significant source of physiological stress to the animals. Within the sample size available to date, it appears that capture and restraint without anesthesia elicit a greater physiological stress response than any procedure conducted under gas anesthesia. For example, rehabilitation procedures such as those carried out at The Marine Mammal Center during the rehabilitation of beached pinnipeds, result in higher fecal glucocorticoid levels ("stress hormones"), than any of the observed surgical procedures. This does not mean that surgical procedures do not result in stress. It does however indicate that these procedures are not more stressful - and likely less so - than routine rehabilitation procedures. These findings are summarized in the following publication submitted to the Journal of Experimental Zoology, and under review:

> *Petrauskas L, Atkinson S, Gulland FMD, Mellish JE, Horning M. In review. Monitoring glucocorticoid response to rehabilitation and research procedures in California and Steller sea lions. Submitted to Journal of Experimental Zoology.*

Very briefly, in the above referenced manuscript we report results from a study on 34 California sea lions and six Steller sea lions. Of eleven animals that underwent invasive surgery, only one exhibited slightly increased fecal corticosterone levels in response to surgery. None of the study groups, including groups of physical restraint only (n=9), gas anesthesia only (n=10), and various levels of surgery (n=15), exhibited significant changes in fecal corticosterone levels. Capture and restraint elicited greater responses (but still not significant) than surgical procedures.

In addition, our recent studies have shown that animals that have received LHX tag implants, and have also received external devices prior to release, do not exhibit post-release movements and behavior that are any different than non-implanted animals. This suggests that carrying implants and external tags simultaneously does not increase the animal's 'burden' in a detectable way. This does not mean there is no effect on the animals, however, compared to natural factors contributing to variance in observed parameters, these effects are negligible. Findings are summarized in the following publication submitted to the Journal for Experimental Marine Biology and Ecology, and under review:

Thomton J, Mellish JE, Horning M. In review. Dive behavior and movement patterns of juvenile Steller sea lions after intra-abdominal transmitter implantation. Submitted to Journal of Experimental Marine Biology and Ecology.

In summary of this manuscript (from response to application #881-1890 comment #46, ASLC, with permission):
"LHX implant recipients - During the 91.5 ± 8.6 day tracking period following release from temporary captivity, all sea lions returned to their respective capture haul-outs. Dive depth, duration, frequency and dispersal distances were similar to non-implanted animals. A possible captive recovery effect was observed during the first week post-release when dive depths were shallower and durations shorter than free-ranging juveniles. By the second week all dive behavior parameters were within the normal range and did not differ from non-implanted animals. This effect was also observed in non-implanted individuals maintained in temporary captivity. In conclusion, surgical implantation of LHX tags does not appear to have a negative effect on the short-term dive behavior or movement of juvenile sea lions."

17. It is worth noting, however, that this risk-prone surgical procedure which the applicant proposes to utilize in remote areas of Alaska was prohibited by NMFS in 2005 outside of the ASLC facility because NMFS wished to assure that "animals could be monitored by veterinary and husbandry staff for several days post-operatively and treated should there be any complications from the surgery." (EA at 25) This procedure remains risky and we do not feel that it should be used in the field where animals cannot be monitored postsurgery.

We are not aware that this procedure was "prohibited outside of the ASLC facility", but the procedure was not (yet) authorized outside of that facility.

When developing the experimental design of the LHX project, the design included a three-stage approach to the full implementation of the project. Stage 1 was the application on a surrogate species, followed by Stage 2, the application on the target species under controlled condition (at the ASLC facility), and then in Stage 3 the full implementation of the project using the projected sample size. These three stages are to be carried out successively but with some overlap, and the originally projected sample size for Stage 2 was 12 to 16 animals. We would not proceed to Stage 3, until a minimum number of animals are successfully completed under Stage 2, likely 8-10 animals, depending on how well the animals do under these conditions.

To date, the 'success' of the implantation procedures (if defined as successful release following captive observation period, without complications) on two species, is 10/10. Therefore, increasing sample sizes of stages 1 and 2 cannot further increase success rate. The success rate can only decrease. While this is not likely given the risk factor assigned to LHX procedures in the PEIS, the bottom line is: the more such procedures you do, the greater the likelihood of complications (just as in human health care surgeries in

hospitals). Thus, beyond a given sample size, a continuation of 'validation studies' is counter productive.

The risk does not differ between procedures conducted at the ASLC and in the field, since methodologies and setups (surgical container) are identical. What does differ is the ability to subsequently monitor animals, and take corrective measures in case of complications. The likelihood of complications is exactly the same. Based on PEIS risk assessment probabilities assigned to these procedures, it is most likely that even stages 2 and 3 of the LHX project combined will not result in a single unintentional mortality.

We would also like to note that the success rate to date of 10/10, does support the estimated risk probability used in the PEIS.

18. We think it is commendable that the applicant seeks to assure that animals subjected to branding are provided with anesthesia, and he makes a sound case for its use. He also incorporates controls to prove the reliability of the methodology (e.g., implanting the device in carcasses to test retrievability, using duplicate implantation to assure that there is reliability in data retrieval). Having said this; however, we are concerned that this technology may not yet be appropriate for use with this species. The applicant states that six juvenile Steller sea lions were implanted with these devices prior to the permits being vacated by court order in 2006. His assertion that no tags have transmitted data (something that can only happen upon the death of the animal) means that all animals are still alive may or may not be true. He himself states that there is a concern with tags being released in rocky or other areas that may prevent or occlude signal transmission.

The intent of carcass testing is not to prove the reliability of the methodology. The intent of the carcass testing is the determination of the effect of the statistical non-independence of dual LHX tags, on failure rate calculations based on dual tag returns.

Some tags may fail or be unable to transmit. This is taken into account in the experimental design of the LHX project, through the deployment of dual tags. While dual tag deployments have the added benefit of raising data recovery probability, the primary reason for dual deployments is the failure rate assessment, for which dual tags are essential.

Since the permit application was submitted, we have received LHX tag data returns from one of the six juvenile Steller sea lions that had received LHX tags. The data transmitted through the Argos system, and recovery circumstances, strongly suggest that the animal died from external trauma. The most likely cause of external trauma is deemed to be predation. However, this is as yet a preliminary conclusion, since data analysis and testing are continuing.
The returned data allows the following preliminary conclusions:

a) *LHX devices allow the effective monitoring of individual animals throughout their lives, and allow an assessment of likely death causality.*

b) *In the particular instance of the first LHX data recovery, death was likely by external trauma. This conversely confirms that until the external trauma event, the animal was alive and well, more than one year after the date of implantation. Thus, this confirms in this single case that LHX tags did not affect survival of this juvenile Steller sea lion.*
(see also comment #1 by the Marine Mammal Commission).

c) *Within the limited sample size available to date, available data returns within the present monitoring period support the risk assessment used in the PEIS, for LHX procedures.*

19. Further, there is uncertainty regarding sampling and use of various protocols. The capture location is said to be determined "3-6 months before field work commences" (page 17) and the permit applicant proposes to coordinate sampling with NMML, ADF&G and ASLC. Yet we note that the NMML permit application does not propose more than a few weeks notice of the location of sampling sites.

This is a matter of coordination. The ASLC, NMML, and ADF&G are all participants in the LHX project, and as such coordination is standard procedures for all parties. It is stated in the permit application that "The planning of such research will be done in consultation with Steller sea lion investigators at the National Marine Mammal Laboratory (NMFS), the Alaska Dept. of Fish & Game (ADF&G), the Alaska Sea Life Center (ASLC) and other principal investigators that hold the major Steller sea lion field research permits."As stated in the NMML permit application, the notification time frame is simply "a standard notification condition included in all Steller sea lion research permits" *Dr. Horning is listed as a co-Investigator of the ASLC application (#881-1890), and Dr. Mellish (ASLC), Dr. Gelatt (NMML), and Dr. Rea (ADF&G) are listed as co-I's on this application, and thus information exchange and coordination is not constrained by the time frame of the 'standard notification condition'.*
In terms of sampling protocols, the specific location from which animals will be captured, is irrelevant for our project, affording us a large degree of flexibility in terms of coordinating with other projects. As stated in the application:
"Ad-hoc determination of sampling locations and dates is not a problem for our experimental design: as detailed in section B.2. (Background, 'The Problem') our experimental design with LHX tags is based on directly identifying 'problem animals' within the Western DPS using mortality information on individual animals, irrespective of small scale capture location."

20. Moreover, although this applicant wishes to be precautionary and avoid taking animals from a site that "has been disturbed or is expected to be disturbed in the

near future by other researchers," (ibid) it will not be possible to assure this several months in advance if NMFS accepts other permittee proposals with shorter time frames and/or if the information in the DEIS is correct and research sites are not reported by most researchers until after research has already been conducted.

See response to comment #19.

21. The target of this research is juvenile Steller sea lions, the demographic considered most at risk in some hypotheses of the decline. We understand that this is, therefore, the demographic most in need of study; however, the use of a procedure that has only been tried previously on 10 sea lions is risk prone at this time.

See responses to comment #17 above: beyond a given sample size, trying a given experimental design on larger sample sizes does not change the risk assessment.

It is important to also consider the combination of risk estimates for given types of procedures, combined with the requested sample sizes. The risk for LHX implant procedures on its own may be higher than for some other procedures. However, the requested sample size is very low, for a demographic project.

The risk assessment for this project in the PEIS suggests that the most likely outcome of this project is that in 100 animals, not a single unintentional mortality will occur: the factor of risk for individual procedure, multiplied by sample size, suggest a likely number of unintentional mortalities of 0.1 for a sample size of 100 animals. However, it is impossible for 0.1 animals to die. Therefore, the statistically correct statement in this context is that with 90% probability no unintentional mortalities will occur as a result of this project. The risk probability used for the PEIS is an estimate, but the success rate to date of these procedures (see responses to #17), does support this risk probability estimate.

22. The applicant seeks incidental mortality of up to 5 animals per year. If we assume that 20 per year will be captured (as it says on page 1and elsewhere in the application) then the mortality rate would be as high as 20% per year. Incidentally killing one out of every five animals is unacceptable. We wonder that this applicant's IACUC approved such a high death rate. We would prefer to see this methodology tried and shown to be risk averse in additional surrogate species such as additional trials in California sea lions (or Steller sea lions from the Eastern stock) before it is permitted for use with animals from the endangered Western stock, which is still declining and showing reduced juvenile survival in many of the proposed sampling areas.

Animal Use Permits issued by IACUCs are not approving or disapproving mortality rates, in particular 'partial rates'. Instead, AUPs authorize specific maximum numbers of unintentional mortalities, and these are approved in relation to the requested sample size for the entire project. Actual unintentional mortality rates for a given methodology should only be calculated for the entire project, and not for a portion of the project (or sub-set of the overall sample size) that yields a theoretically maximum rate.
To use a ficticious example, it would be false to report a rate of zero, if during the last year of a project no unintentional mortalities occurred in e.g. 50 animals, when e.g. one may have occurred in the previous year. The correct rate would not be 0% (last year only), nor 2% (first year only), the correct rate would be 1% (entire project), in this example.

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.

    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.

    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.

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

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.

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.

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*).

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. salvia, 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

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.

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.

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.

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.

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.

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.

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.

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 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.

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.

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.

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, 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.

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, forceable 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?

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. salvia, 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.

Literature Cited:

Greig, D.J., K. Mashburn, M. Rutishauser, F.M.D. Gulland, T. Willliams, and S. Atkinson. 2007. Seasonal changes in circulating progesterone and estrogen concentrations in the California sea lion (*Zalophus Californianus*). Journal of Mammalogy 88: 67-72.

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

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

Mashburn, K.L., and Atkinson, S.A (2007). 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.

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.

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.

National Marine Fisheries Service (NMFS). 2007. Steller Sea Lion and Northern Fur Seal Research. Final Programmatic Environmental Impact Statement. National Marine Fisheries Service, Silver Spring, MD. 1110 pages.

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.

Williams TM, M Rutishauser, B Long, T Fink, J Gafney, H Mostman-Liwanag, D Casper, *In press.* Seasonal Variability in Otariid Energetics: Implications for the Effects of Predators on Localized Prey Resources. Physiological and Biochemical Zoology.

Table 1- Alaska SeaLife Center pinniped holding facilities: Pool surface area (SA), pool depth, and land surface area (DRA) are used to calculate maximum number of animals that could legally be housed within an area while complying with USDA APHIS regulations.

Pool	SA (ft^2)	Depth (ft)	DRA (ft^2)	Adult Male SSL	Female SSL	Sub-Adult Male SSL	
SSL Habitat East	883	16.7	2000	1 or	12 or	7	1 sexually mature male and 11 females or 9 sub-adult males or some combination of sub-adult males and females
HS Habitat	371	17.5	650		5 or	4	4 sub adult males or 5 females or a combination of both
SSL Holding	244	7	261	1 or	3 or	2	2 sub adult males and 1 female or 1 sub adult male and 2 females or 3 females
154 A	133	6.6	146		2 or		2 females
154 B	133	6.6	146		2 or		2 females
ODL 5	314	6	572	1 or	5 or	3	1 sexually mature male and 2 females or 3 sub adult males or 2 sub adult males and 2 females or 5 females
ODL 6	314	9.5	572	1 or	5 or	3	1 sexually mature male and 2 females or 3 sub adult males or 2 sub adult males and 2 females or 5 females
ODL 7	134	6	163		2		2 females
ODL 8	1396	9	1425	1 or	22 or	15	1 sexually mature male and up to 20 females or up to 15 sub adult males or some combination of females and sub adult males
SSB 1	133	4.8	464		2 or		2 females
SSB 2	214	4.8	383		3 or	2	3 females or 2 sub adult males or 1 sub adult male and 2 females
SSB 3	214	4.8	383		3 or	2	3 females or 2 sub adult males or 1 sub adult male and 2 females
SSB 4	214	4.8	383		3 or	2	3 females or 2 sub adult males or 1 sub adult male and 2 females

Table 2- 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) or 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			

Comments received on File No. 881-1893 (Alaska SeaLife Center, northern fur seal research)

Marine Mammal Commission

1. The application states that the "use of sedatives and anesthetics will only be conducted under the supervision of a veterinarian or an individual that has received training from a veterinary anesthetist and that has significant experience in anesthetizing fur seals." If a veterinarian will not be present in the field, an explanation should be provided. If a veterinarian will be present, his or her curriculum vitae should be submitted if it is not already on file.

Response: A veterinarian, most likely one of the Alaska Sealife Center staff veterinarians, will be on hand for all procedures

USDA APHIS

2. No consideration was given to proper recovery times for anesthesia. While an animal may be awake and mobile within 20 minutes of cessation of isoflurane, it is a recognized occurrence in veterinary medicine that the effects of anesthesia do not dissipate after 20 minutes. In practice, we have seen animals take up to 24-48 hours to recover from anesthesia, especially when the procedure of lengthy (over an hour).

PR1: Please explain what considerations are made for recovery of animals from anesthesia versus the initial return to consciousness.

Response: Our experience has been that fur seals recover quickly from isoflurane anesthesia even when anesthetized for as long as 2.5 hours if no pre-sedation is given. Fur seals are easily handled and restrained for masking, so we anticipate that most if not all fur seals will be anesthetized with isoflurane only. Fur seals will be closely monitored throughout the anesthetic recovery process from the initial return to consciousness through the ability to move and react normally. The seals receive oxygen for 5 to 10 minutes after isoflurane is discontinued, at least until they regain muscle tone and the endotracheal tub is removed. The anesthetist remains in immediate contact with the animal until it is extubated. A physical barrier will be used to prevent other animals from approaching a recovering seal until it is moving normally and is capable of swimming and defending itself from conspecific aggression.
The animal must be fully recovered, very alert and ambulating normally before they leave the recovery area. Consciousness alone is not the only criteria for release; seals must be alert, responsive and maintaining muscle coordination.

3. While small surface skin biopsies may be acceptable without anesthesia under some conditions, proposals for blubber and muscle biopsies, some up to 2 ½ inches deep, constitute painful and invasive procedures and must be done under anesthesia. If local anesthesia is to be used, dosages should be given, and well as documented protocols for determination of effectiveness, including the waiting period for full effect.

PR1: Please provide details about use of anesthesia for blubber or muscle biopsy, including dosages, how you determined the specific anesthetic/dosage would provide

appropriate level of anesthesia, and how long you would wait post-delivery for the anesthetic to take effect. If you do not intend to use anesthesia for these procedures, please explain why.

Response: All muscle and blubber biopsies will only take place while the animal is under general gas anesthesia, which provides complete pain relief for these procedures.

4. The amount of blood expected to be taken at sampling, while below 10% of blood volume, are significantly high. Given the state of current laboratory methods, it seems that samples can be much smaller, as most tests no longer require 5 ml of serum anymore, more like 0.1 ml. Remember refinement and reduction – this can be applied to sampling as well.

PR1: Please explain why your specific studies require collection of the amount of blood requested in your application.

Response: Blood collection is limited to 2% total body mass (e.g., 2 ml/kg body mass) and is well below accepted veterinary standards (e.g., Murray 2000), as noted in our application. The APHIS reviewer is correct in that some clinical lab tests, such as a comprehensive profile, can be run on as little as 0.1 ml of blood. However, we are making the most of the invasive manipulation of drawing blood in order to learn as much as possible about the health, condition, and potential disease states of the sampled fur seals. The analytical tests that we plan to conduct are usually run in at least duplicate, if not triplicate to assure accuracy.
Estimated volumes of blood for required analyses:
Blood biochemistry: 5 ml blood
Hematology: 1 ml blood
Serology: 5 ml blood
Endocrinology: 5 ml blood
Immunology: 5 ml blood
Contaminants: 5 ml
Archive: 5 ml
The archive will be collected in case a vial meant for a particular assay is broken or if there is an error that requires an assay to be re-run.
In our application we stated all the different assays that we planned to run: "Blood will be collected and analyzed for hematology and blood chemistry (including glucose, lactate dehydrogenase, creatine phosphokinase, blood urea nitrogen, triglyceride, non-esterified fatty acids, creatinine, sodium, potassium, chloride, calcium, phosphorus, alkaline phosphatase, albumin, gamma-glutamyl transpeptidase, serum glutamate pyruvate transaminase, total protein, cholesterol, globulin, total bilirubin), for serology, bateriology and virology (includes brucella, leptospirosis, seal herpesvirus, toxoplasmosis), endocrinology (including assays of free and bound triiodothyronine and thyroxine, cortisol, corticosterone, testosterone, progesterone, estrogen, leptin and ghrelin). Blood may also be analyzed for contaminants, including organochlorines, PCBs, DDTs, mercury, pesticides." We are aware that the reduction of blood sampling

volumes to the minimum required to achieve analytical and archival needs is an important and necessary factor to ensure animal welfare, and we have done so appropriately.

5. As discussed above, all should address the potentially painful procedures and care of the animals.

PR1: Please explain measures that would be taken during and after potentially painful procedures.

Response: A*ll procedures are subject to rigorous internal review by the Alaska Sealife Center IACUC and all protocols that may involve pain or distress have been scrutinized. All procedures listed in the application were reviewed and approved by Alaska Sealife Center IACUC this year. The IACUC AUP approval letter will be forwarded to PR1 upon granting of the permit.*

6. Applicants identify that studies have already proven that certain methods are equivalent to the "gold standard" –deuterium(?) measures – for determining body composition. Therefore, it appears to be redundant and unnecessary for more than one method be used, increasing the handling and sedation/anesthesia. Only one method for determination of body composition should be used for each study. The intent of the studies is to monitor the animals, not compare methodologies. As an endangered species, these sea lions should be studied, not experimented on.

PR1: Please explain why it is necessary to use multiple methods on the same animal for determining body composition and why comparison or validation of the methods cannot be performed using a non-ESA listed species.

Northern fur seals are NOT an ESA-listed species. However, even if they were, we would have to run these validations on northern fur seals because the correlations are species specific.

Although the gold standard for determining body composition is isotopic water dilution, it requires repeated blood sampling over a 2 hour period, as explained in our application. There are at least two other methods of body composition analysis that are much less invasive, but neither of these provides an absolute number for total body water (and by calculation percent body fat) as does the labelled water method. These methods are bioelectric impedance analysis (BIA) and ultrasonic imaging of subcutaneous blubber thickness. Although these latter two methods take less time to perform and may therefore have less impact on seals, predictive equations for relating the reactance and resistance values obtained by BIA to percent body water or percent lipid do not exist for northern fur seals. Although we expect to find a reasonable correlation based on studies with other otariids, we must first demonstrate that the BIA values actually correlate well with a measure of body condition such as percent lipid in the northern fur seal. There is also a lack of predictive equations for northern fur seals to convert subcutaneous blubber thickness to percent body lipid. It is prudent to conduct a species specific validation trial

before relying on an unvalidated technique because of species differences in tissue hydration states and lipid compartmentalization. Arnould (1995) has demonstrated the utility of bioelectric impedance analysis on adult female Antarctic fur seals, but there are no studies of its validity when used on young fur seals of any fur seal species. Therefore, we will perform all three measurements on the same individuals. These species-specific studies will then be published so that we and others can use the correlation equations to predict body composition using the better of the two methods that require much less handling time.

7. Discussion of threshold levels for mortality and stopping need to be addressed. It is unclear what that level is. Level may be same or different for level A and level B harassment activities. Express the results as real numbers, for example, the studies will be stopped if 1 level A animal dies, or if 3 level B animals die. These are not suggestions for the levels, but examples.

Response: For our past permits, these levels were set by the Office of Protected Resources as a condition of our permit. Perhaps OPR can provide some clarification on this point.

8. The institution needs to address alternatives to biopsy techniques proposed, and if muscle biopsies are needed. There is no justification presented for this sample. All prior comments apply to this application as well.

Response: On page 15, of the application, we explain why we are collecting muscle biopsies: "Muscle enzyme profiles will be examined to determine the aerobic condition of fur seals prior to their commencement of migration. The aerobic condition of fur seal pups may be an important determinant of their diving ability and therefore foraging success and ultimate survival." There is no less invasive way of determining the muscular aerobic potential of a fur seal.

9. Use of sedation and anesthesia must be under the direct supervision of a qualified veterinarian. Valium is a controlled substance and can only be prescribed and dispensed for a specific patient by a veterinarian.

PR1: Please clarify whether or how a qualified veterinarian is involved in the proposed activities.

Response: The administration of controlled substances will only be conducted by a qualified veterinarian.

10. Tooth extraction (this can be applied to all applications as well) has not been justified, as there are no aspects of the study that require precise aging. The categories referenced are broad and the experienced and trained researchers should be able to tell approximate age without having to pull a tooth and the dangers the procedure entails (anesthesia, malocclusion, dental abscesses, pain).

PR1: Please explain why your study requires the precision age determination associated with tooth extraction rather than relying on other methods for grossly estimating age.

Response: We are unaware of any methods, certainly none that are published, for precisely aging northern fur seals in a way that is less invasive than pulling a tooth. Fur seals will be anesthetized in order to facilitate many different procedures, so anesthesia is not performed just to pull a tooth, i.e., there are many other justifications for anesthetizing the captured seals. The color of facial vibrissae can provide only a gross index of age. Facial vibrissae are black at birth and remain black through age 3 years, but become mixed (black and white) at approximately 4 to 5 years of age; and by age 7, the vibrissae usually are entirely white. However, in order to quantify the demography of the population, you need much more precise age estimates than that. For example, in many species reproductive performance varies with age, so when we capture fur seals at sea and use ultrasonography to determine pregnancy, it is critical to be able to assign an age to the sampled seal. Goebel et al. (2005) examined the long and short-term effects of non-lethal tooth extraction on Antarctic fur seals, and the only short-term effect was a minor effect on maternal attendance (on-shore visit duration was slightly longer after tooth extraction). Tooth extraction had no effect on over winter survival, fecundity, mass gain or diving behavior (Goebel et al. 2005).

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11. I suspect blood collections from the jugular vein are unnecessary and should be avoided whenever possible.

PR1: Please explain why you propose this method of blood collection and how it meets the MMPA's humane standard for your application.

Response: Fur seals that are captured at sea in the Gulf of Alaska may have a slightly reduced body temperature when blood collection is attempted, and therefore blood withdrawal from veins in the flippers may not be possible due to peripheral vasoconstriction. Venipuncture from the jugular vein is no different from venipuncture of the caudal gluteal veins, so it is quite humane. As a matter of fact, the jugular vein is a large vein that can be localized by an experienced person, whereas the caudal plexus veins are smaller, are surrounded by nerves, and are localized by using approximate landmarks. For this reason, we limit ourselves to no more than 3 punctures when trying to draw blood from the caudal plexus. If we fail to draw sufficient blood from this region, rather than continuing to puncture the same area and potentially causing hemorrhage or nerve damage, we will attempt venipuncture of the jugular vein. Gulland et al. (2001), describe venipuncture of the jugular vein as an acceptable method for otariids in the CRC Handbook of Marine Mammal Medicine.

12. Dr. Andrews described hypotheses useful to NMFS in assessing implementation of research components of the northern fur seal conservation plan. NMFS would like more details regarding how aspects of the proposed northern fur seal pup research complements results from very similar research implemented by the National Marine Mammal Laboratory in 2005 and 2006.

NMFS encourages research to last long enough to reasonably quantify inter-annual variability, and feels that recent NMML investigations have likely quantified pup migration during the first year of independence. We encourage Dr. Andrews to implement studies that complement and build upon recent work.

Response: The studies of weaned pups performed by NMML are quite valuable and as explained in our application we hope to provide additional information about the foraging behavior of weaned pups that could not be gained in those previous studies. For example, they could not determine when or where the fur seals were capturing prey. By using satellite-linked stomach temperature measurements, we will be able to determine the time and three-dimensional location of prey capture by fur seal pups. By combing environmental data with specific information of the foraging success of weaned pups, we will be able to map the pelagic movements, diving behavior and foraging success of fur seal pups in association with hydrographic features to characterize three-dimensional habitat.

13. NMFS suggests Dr. Andrews provide adequate justification for the use of stomach pills (item i) and how this more invasive procedure will provide data useful to conservation and management needs. NMFS would like more information regarding how Dr. Andrews will differentiate successful and unsuccessful foraging. Do unsuccessful attempts not result in seawater entering the stomach and thus lower stomach temperature? If there is information confirming stomach temperature drops are always indicative of successful foraging that would be important to describe. To our knowledge comparable foraging success data do not exist for adults or juveniles, thus conclusions and management actions that could result from the proposed work will be of very limited application.

Response: We feel that our application provides ample justification for the use of stomach temperature telemetry. Satellite transmitters that only provide information on the movements or the dive behavior of a seal do not provide information on what areas were successful foraging areas and which ones were not. The draft northern fur seal conservation plan points out that the ability to understand fur seal foraging ecology is constrained by the inability to identify successful foraging (NMFS 2006a, pg. 54), and that conservation measures and management actions are complicated by this constraint. Stomach temperature can provide an indication of successful foraging that can then be used to determine which areas are most productive for fur seals and which therefore might deserve special protection. The preliminary results from the NMML tracking studies of weaned fur seal pups show that these pups spread out over most of the North Pacific. We aim to correlate movements and foraging success with environmental features in a way that might permit the identification of habitat that is truly "critical". The utility of stomach temperature telemetry has been recognized by the Steller sea lion Recovery Team and its use is recommended in the most recent Steller sea lion Recovery Plan (NMFS 2006b). The stomach temperature drop rate, absolute temperature drop, and recovery time can be used to distinguish water ingestion from prey ingestion (Wilson et al. 1995; Andrews, 1998; Kuhn and Costa, 2006). The reviewer's claim that

comparable data from juveniles and adults are needed before our data on weaned pups can be of value is unsupported and we definitely do not agree.

14. The contention that penguins are "more" streamlined than northern fur seal pups and thus drag effects will be much less is unsupported, and if data or studies support such a statement, please have Dr. Andrews provide them.

Response: Although the coefficient of drag for northern fur seals has not been measured directly, they have a similar body shape and fineness ratio as California sea lions. California sea lions (Feldkamp, 1987) have a much higher drag coefficient and cost of transport than pygoscelid penguins (Bilo and Nachtigall, 1980; Culik et al. 1994).

15. NMFS would also prefer an examination and discussion of the proportional weight and drag increase of recently weaned pups to better understand how the proposed work will influence survival.

Response: The instruments that we will use are almost identical in size and shape as those (SPLASH tags) used in 2005 and 2006 by NMFS researchers.

16. Dr. Andrews notes that researchers or veterinarians with "sufficient experience" will biopsy northern fur seals and conduct other work. NMFS feels a definition of "sufficient" is necessary to ensure the effects of the proposed work are mitigated to the greatest extent practical. A definition such as "worked under the supervision of an experienced veterinarian or researcher on at least 10 procedures on northern fur seals or other fur seals" may be a reasonable standard.

Response: This information was already provided on page 13 of our application: "We consider that personnel who have received the appropriate training (i.e. they have received instructions from an experienced person and had previously observed a minimum of five events) and have correctly performed the procedure under the supervision of a qualified researcher or veterinarian, to have "sufficient experience."

17. NMFS suggests differences in skin and pelage between sea lions and fur seals make assumptions about minor effects and reasonable techniques highly speculative. Currently there are captive northern fur seals at The Marine Mammal Center (TMMC) in Sausalito, CA. NMFS believes testing new techniques and gaining "sufficient experience" are best accomplished on captive animals in controlled circumstances. Dr. Andrews proposes a number of new techniques the NMFS feels are better applied in more controlled circumstance with captive northern fur seals. Collaborating with the TMMC to examine body composition with isotopic water dilution, bioelectric impedance analysis, and ultrasonic imaging would be more appropriate and provide the opportunity for replication on known individuals.

PR1: Please discuss why you would not use surrogates (e.g., animals at TMMC or other captive animals) for testing these types of procedures and gaining sufficient experience.

Response: In both the application and in response to comment #6 we explain the reasons for performing a species specific validation and determination of correlation equations for body condition methods. Furthermore, we will perform these validations on seals that we have already captured and anesthetized for other purposes (such as scientific instrument attachment), but if we used other fur seals, such as seals at The Marine Mammal Center, our total take of animals would go up, which is exactly the opposite of what NMFS should be encouraging us to do. Also, none of the techniques that we propose to use are novel. The reviewer should not confuse the need to determine species-specific correlation equations with "novelty". The NMFS reviewer should be familiar with NMFS' own use of many of the methods that the reviewer mentions above. For example, ultrasonography to determine pregnancy was first used on northern fur seals by NMFS researchers (W. Testa, personal communication). Isotopic water dilution is an important method in a study being conducted on northern fur seals by NMFS researcher Rolf Ream and UAF collaborators A. Springer and A. Banks. Although recent use of stomach temperature telemetry in northern fur seals has not yet been published, it has been used extensively in other pinnipeds (c.f. Gales and Renouf, 1993; Andrews, 1998; Austin et al. 2006; Kuhn and Costa, 2006) and at least 25 species of seabirds (c.f. review in Wilson et al., 1998).

> 18. In addition the effects of extracting vibrissae seem to be downplayed with no discussion of the potential for infection or trauma to sensitive facial tissue on pups. It would be useful for Dr. Andrews to better describe the size and number of pup vibrissae necessary to examine the mother's trophic foraging level. NMFS suggests items (e) and (g) should also be tested and confirmed on captive animals rather than wild animals where inference and interpretation are highly uncertain. At a minimum, the proposal for extraction of vibrissae should be reviewed by a NMFS veterinarian.

Response: Our protocol for withdrawing whiskers is available for review. We will pull only a single whisker from pups, which will provide sufficient material for stable isotope determination (Hirons et al. 2001).

> 19. NMFS is concerned about the risks associated with stomach lavage (item k), and would like a more thorough discussion of the procedure and its implementation at sea. The use of disposable materials would mitigate some of the cross-contamination risk but it is unclear if disposable materials are available.

Response: We will be using the method of stomach lavage that has been used safely and effectively on pinnipeds and seabirds (c.f. Pierce and Boyle, 1991; Votier et al 2003). Our application clearly states that the equipment will be thoroughly

disinfected between uses on different animals, which is standard veterinary practice for stomach tubes.

20. NMFS suggests Dr. Andrews reconsider implementation of novel methods in the wild and instead practice and validate analyses on captive fur seals at institutions and facilities such as The Marine Mammal Center, followed by informed field investigations.

Response: As explained above in response to comment 17, we are not proposing to use any novel methods in this study.

21. Dr. Andrews down-plays individual and stock effects and a more thorough description and quantification of effects is necessary to evaluate whether mitigation is adequate. A power analysis is critical to confirming whether sample sizes are adequate to test the proposed hypotheses regarding adult versus pup migration, diving behavior, and habitat associations. Potentially more important than the power analysis is to develop hypotheses and studies to build on and complement recent work describing winter pup and adult migration along with habitat associations.

Response: A power analysis was conducted and was used to arrive at the sample size we chose. We agree with the reviewer that hypotheses are important, and this is why we clearly stated hypotheses in our application. The reviewer's statement about "down-playing" the individual and stock effects is not accurate. We are very concerned about the effects of our research on both the individual and the stock in question. We are proposing to use the least invasive techniques available to gather the data needed to address our hypotheses and the welfare of the study subjects is our utmost concern. However, it is clear that even if we have grossly underestimated the potential for adverse effects and mortality of individuals, our project could not possibly have a measurable effect on the population. Our sample size of 250 captures is only 1.6 percent of PBR for the eastern Pacific stock of northern fur seals.

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22. It is clearly important to understand the questions being investigated by this permit --where animals are dispersing, how they use habitat and what role habitat sufficiency or interactions with commercial fisheries may play in the ongoing declines. There are clear hypotheses being tested. It would be helpful to explain how some of the procedures proposed for captured animals relate to the hypotheses being tested. (e.g., how some of the invasive sampling protocols specifically relate to investigating the three hypotheses regarding habitat use described on pages 6 and 7 of the application). If these procedures are not clearly enlightening the questions being informing the testing of these hypotheses, they may be subjecting animals to unnecessary additional stress or potential for harm.

Response: We have no intention of subjecting animals to unnecessary stress or harm. This is one of the main factors that is reviewed by our IACUC, and this project has been

reviewed and approved by the Alaska Sealife Center IACUC. Our application provides justification for each procedure. The final sentence of the HSUS' comment is unclear.

> 23. We appreciate the applicant's candor in admitting that the actual number of mortalities that may result during capture, sedation and restraint is not clear and the admission that it may be higher than the number of mortalities stated (0.08). The applicant states that this "seems a reasonable threshold above which research activities would halt until a review can be conducted." It is not clear what is meant by this statement, but an 8 percent mortality rate is quite high in comparison to that projected in the DEIS (see 4-52, 4-53).

Response: The HSUS has mis-read our application. On page 11 of our application, we clearly said "0.08 percent". The HSUS has multiplied our estimate by 100, but we clearly stated that the value 0.08 was the "percent". They could have done the math themselves and found that 0.08 percent of 5000 is 4, the number of mortalities that we requested we be permitted in the case of accidental mortalities.

> 24. The sample size (50 pups and 200 pelagically captured fur seals of mixed ages) was determined by what the applicant felt could be logistically handled. One hopes that this sample size is sufficient to collect data sufficiently robust to address the questions being asked.

Response: Our sample sizes were chosen based on power analyses, although we admit that the amount of variation for parameters such as distance moved or time spent in finite areas by weaned pups was dependent upon data from other species because there are no published data to which we could refer for northern fur seal weaned pups.. For adequately describing habitat use, a sample size of 50 should be adequate, based on a study in which southern elephant seals were instrumented with satellite tracking devices (Hindell et al. 2003). That study demonstrated that at least 25 animals were necessary to provide a useful representation of habitat use, but that nearly 95% of the actual area used would be identified with a sample of 40 individuals. By adding the additional variable of foraging success, we might require a larger sample size, but we are confident that with our sample size of 50 weaned pups we will gain extremely valuable data and insights.

> 25. As noted previously, the various intrusive procedures being used on animals of mixed aged animals being captured at sea do not appear clearly related to the hypotheses outlined on page 6-7 (i.e. how will the use of fecal loops, bioelectrical impedance analysis and other such procedures illuminate the three hypotheses that: fur seal pups migrate to the same areas as adult females, that the diving behavior of pups is dissimilar to adults or that there are correlations between distribution and physiographic and hydrographic features that may concentrate zooplankton and micronekton stocks?). In fact three of the procedures (i.e., the of bioelectric impedance, ultrasonic imaging and isotope dilution) are being used redundantly, largely to correlate/validate their results with one another in measuring the same variable of body condition.(page 11) This is not part of any

hypotheses being proposed. This may be a worthwhile study, but it was not part of the initial description of the purposes of this permit. Further, although stomach temperature telemetry has been used on Steller sea lions, its use and effects on fur seals is not known. If the safety and efficacy of the use of this technology is part of what is being tested, it should be so stated in the permit.

Response: We have addressed similar concerns about the use of three different methods of measuring body composition in our response to comments #6 and #17. As we clearly stated in the application, once we generate the species specific equations, we and others should be able to use a less invasive technique than labeled water dilution to predict total body water and therefore stores of body fat. We are not testing the safety or efficacy of our methods, as these have already been demonstrated in other studies. Stomach temperature telemetry has been used extensively in other pinnipeds (c.f. Gales and Renouf, 1993; Andrews, 1998; Austin et al. 2006; Kuhn and Costa, 2006) and at least 25 species of seabirds (c.f. review in Wilson et al., 1998).

26. The applicant's response to the form's NEPA considerations requires expansion. One response, involving identification of new or experimental protocols in the permit application, should more clearly discuss the proposed evaluation of the correlation between various tools to evaluate body condition (as discussed above) as well as the fairly novel use of stomach temperature sensors in this species.

Response: See response to comments #6, #17, and #25. We are not proposing to use any novel techniques in this project.

27. The application indicates that some animals will receive sedation or anti-anxiety drugs and some will not. We believe that this should be consistent. We are also concerned that anesthesia may be administered by personnel without significant qualifications (e.g., page 12 states that they may be administered "*under the supervision* of a veterinarian *or* an individual that [sic] has received training from a veterinary anesthetist…" Thus it appears that a non-veterinarian may be supervising administration of anesthesia by a person with even less training. This is inappropriate. See 50 C.F.R. § 216.34(g) ("Individuals conducting activities authorized under the permit must possess qualifications commensurate with their duties . . , or must be under direct supervision of a person with such qualifications.)

Response: Anesthesia will be administered only by qualified individuals with sufficient experience with the technique and this species. We consider that personnel who have received the appropriate training (i.e. they have received instructions from an experienced person and had previously observed a minimum of five events) and have correctly performed the procedure under the supervision of a qualified researcher or veterinarian, to have "sufficient experience.

28. As noted above, we are not clear as to the relation of some of the various procedures described on pages 13-18 to the hypotheses outlined in the permit.

Response: The application provides justification for each of the procedures that we are proposing to conduct.

29. This permit proposes important questions to be investigated. The applicant should clearly relate all procedures being proposed to the hypotheses being investigated and address some of the uncertainties identified above.

Response: We are pleased to see that the HSUS feels our research proposal addresses important research questions. We feel we have addressed the uncertainties of the HSUS, and each of our research procedures is necessary to achieve our stated objectives.

Literature cited in Reponses:

Andrews, R. D. (1998). Remotely releasable instruments for monitoring the foraging behaviour of pinnipeds. Mar. Ecol. Prog. Ser. 175, 289-294.

Bilo, D. and W. Nachtigall. 1980. A simple method to determine drag. coefficients in aquatic animals. J Exp Biol 87:357–359.

Culik BM, Bannach R, Wilson RP (1994) External devices on penguins: how important is shape? Mar Biol 118:353±357.

Feldkamp SD (1987) Swimming in the California sea lion: morphometrics drag and energetics. J Exp Biol 131:117±135

Gales R, and D. Renouf. 1993. Detecting and measuring food and water intake in captive seals using temperature telemetry. J Wildlife Management 57(3):514-519

Goebel, M. E., J.D. Lipsky, and R.S. Holt. 2005. The effects of non-lethal post-canine tooth extraction on lactating Antarctic fur seals. Poster presentation. Page 107, In: Abstracts of the 16th Biennial Conference on the Biology of Marine Mammals. Society for Marine Mammalogy, San Diego.

Gulland, F.M.D., M. Haulena, and L.A. Dierauf. 2001. Seals and sea lions. Pages 907 – 926 in Dierauf, L.A. and F.M.D. Gulland (eds.), CRC Handbook of Marine Mammal Medicine. CRC Press, Boca Raton.

Hindell, M.A., Bradshaw, C.J., Sumner, M.D., Michael, K.J., and Burton, H.R. (2003). Dispersal of female southern elephant seals and their prey consumption during the austral summer: relevance to management and oceanographic zones. J. Anim. Ecol. 40:703–715.

Hirons, A., D. Schell, D. St. Aubin. 2001. Growth rates of vibrissae of harbor seals (Phoca vitulina) and Steller sea lions (Eumetopias jubatus). Canadian Journal of Zoology 79: 1053-1061.

Kuhn, C.E. and D.P. Costa. 2006. Identifying and quantifying prey consumption using stomach temperature change in pinnipeds. 209:4524-4532.

National Marine Fisheries Service. 2006a. Draft conservation plan for the Eastern Pacific stock of northern fur seal (Callorhinus ursinus). National Marine Fisheries Service, Juneau, Alaska.

National Marine Fisheries Service. 2006b. Draft recovery plan for the Steller sea lion (Eumetopias jubatus). Report prepared by the Steller Sea Lion Recovery Team for the National Marine Fisheries Service, Silver Spring, MD.

Pierce, G.J. and Boyle, P.R. 1991. A review of methods for diet analysis in piscivorous marine mammals. Oceanogr. Mar. Biol. Ann. Rev. 29: 409-486.

Votier S.C., S. Bearhop, A. MacCormick, N. Ratcliffe and R.W. Furness. 2003. Assessing the diet of great skuas, Catharacta skua, using five different techniques. Polar Biology 26: 20-26

Wilson R, Peters G, Regel J, Grémillet D, Pütz K, Kierspel M, Weimerskirch H, Cooper J. 1998. Short retention times of stomach temperature loggers in free-living seabirds: is there hope in the spring? Marine Biology 130:559-566.

Reply to Reviewer Comments on Permit No. 715-1883
(Andrew Trites, northern fur seal captive research)

May 17, 2007

Marine Mammal Commission (MMC)

1. The application states that all proposed research must also be covered by permits issued by the UBC animal care committee; however, no documentation from the committee has been provided. The applicant should provide such documentation.
   PR1: Please provide copies of any permits and documentation related to such permits issued by UBC animal care committee for this proposed research. If such permits have not been issued, please indicate the status.

   A copy of the UBC Animal Care Permit is attached.

NMFS Reviewers

2. The applicants should be made aware that blood samples are easily obtained (especially as animals get older) via flipper veins. They may wish to consider this technique as it may help to limit the number of needle insertions.

   This alternate sampling spot will be considered (depending on individual flipper formation) as the animals get older.

3. Because the pups have molted and at this time of the year receive considerable thermal insulation from their fur, the applicants could consider marking the pups with bleach rather than clipping the hair.

   While the thermal impact of small shaved spots is probably minimal, we will use bleach marking in lieu of clipping.

4. The tables should list the various types of takes that will be conducted under the "Activity" column. For Table 1, this should include fecal loops and marking. For Table 2, this should include ultrasound, D2O injection

   See attached, revised Tables 1 and 2.

5. Table 2 has a typo—8 animals taken for trained morphological measurements, but only 6 animals are to be in captivity.

   See attached, revised Table 2.

6. Dr. Trites should work closely with the NMFS AK Regional office to begin communication with the community and tribal governments to identify local issues and sensitivity.

Personnel at the NMFS AK Regional Office will be consulted on issues of local sensitivity and coordination of work to minimize disturbance and prevent unnecessary duplication.

7. The application states that the Department of Fisheries and Oceans (DFO) is empowered to enforce the requirements of the original ESA and MMPA permits. A permit authorizing the holding of northern fur seals at the Vancouver Aquarium is being sought from DFO. Please provide a certification statement from DFO indicating that the laws and regulations of the government involved allow enforcement of requirements equivalent to the requirements of the ESA, MMPA, FSA, and AWA, as applicable, and that the government involved will enforce such requirements. Please also indicate the status of this permit and provide a copy if issued.

   As the fur seals will be captured outside of Canada, the Department of Fisheries and Oceans (DFO) will only be involved in issuing a transportation permit. When marine mammals are to be transported across provincial or territorial boundaries, authority to transport under the Marine Mammal Regulations is required and permission must be obtained from DFO. The Consortium will not submit a transportation permit request until the dates and mode of transportation has been finalized.

<u>The Humane Society of the United States (HSUS)</u>

8. It is not clear that study of animals in captivity has sufficiently illuminated any of the hypotheses for the Steller sea lion decline such that research on wild animals is less pressing or more focal. Thus it is not clear that it is warranted here. See 50 C.F.R. § 1374(c)(3)(B) (to research on a depleted stock, "the results of the research [must] directly benefit that species or stock, or that the research fulfills a critically important research need").

   The statement that the study of animals in captivity has not illuminated any of the hypotheses for the sea lion decline is incorrect. Captive pinnipeds have contributed a great deal to the scientific evaluation of the causes of the Steller sea lion decline. Consortium researchers have produced 54 publications (see attached list) that have addressed a broad range of topics including the biological value of prey, accuracy in diet reconstruction methods, aspects of bioenergetics, nutrition, haematology and blood biochemistry, bioenergetic and optimal foraging models, costs of reproduction, hormonal responses, and physiological responses to nutritional stress. Research with the captive animals has also led to the development and testing of new techniques and technologies that can be used to study sea lions in the wild. None of this knowledge could have been gained from animals in the wild and has contributed significantly to our understanding of the nutritional requirements of Steller sea lions and the factors that underlie their population declines. We foresee a captive colony of northern fur seals making an equally important contribution to understanding the decline of their species on the Pribilof Islands.

9. There is discussion of the transport of the pups to the Vancouver Rehabilitation Center for a temporary quarantine before moving them to the "Species at Risk Laboratory" (described on page 25 as an off-display area of the Aquarium). The application should specify all requirements of 50 C.F.R. § 222.308(7), (8) for the transport of animals. There are specifics lacking in the proposal (e.g. qualifications of the transport companies, time in transit, etc.) This information should be provided to NMFS before a permit is granted.

   Ideally, transportation would entail charter aircraft. However, we cannot rely on this possibility. Fur seals would be flown from St. Paul Island to Anchorage via PenAir using a SAAB 340 aircraft that has pressurized and temperature controlled cargo compartment for animal transport. PenAir has extensive experience in transporting animals, and the veterinary team will be in charge of animal health and safety and ensure that all USDA and IATA regulations are met. The direct flight from St. Paul to Anchorage is 3 hr. The animals will then be re-examined before transfer to an Alaska Airlines flight to Seattle (3 hr 30 min). The animals will then be transferred to appropriate ground transportation for the final leg to Vancouver (2 hr 30 min).

10. Page 27 states that the research will take "at least 4 years" and that animals will "become a long-term scientific resource" that is "not suitable or feasible to release back into the wild." Because fur seals live an average of 25 years, (NPUMMRC undated) it seems likely that these animals will become available for display after the life of the experiment.

    The Consortium has endeavored to transfer animals to other facilities (e.g., Alaska SeaLife Center, Mystic Aquarium, Harderwijk Dolfinarium) with active research programs to continue to use these individuals as a scientific resource. In the end, it is probably not feasible nor desirable to reintroduce these individuals back into the wild.

11. There are facilities that already have captive northern fur seals (including Mystic Aquarium). Attempts should be made to partner with facilities holding captive fur seals such that already captive animals can be used for these experiments rather than capturing additional animals from a depleted and declining stock. Vancouver Aquarium (the ultimate destination of 6 of the captured pups) has rehabilitated fur seals in the past and rehabilitation animals also might be more suitable for studies of diet.

    Research will certainly be coordinated as much as possible with facilities (such as the Mystic Aquarium) that currently hold northern fur seals. However, to conduct appropriate research, a group of healthy, uniform (same age, gender) individuals are required to minimize spurious variation in results. Rehab animals can not provide such a group, and are usually precluded by permits from 'rerouting' animals for other purposes.

THE UNIVERSITY OF BRITISH COLUMBIA

ANIMAL CARE CERTIFICATE

Application Number: A06-1432

Investigator or Course Director: Andrew W. Trites

Department: Fisheries

Animals:
Seals Fur Seals 16

Start Date: **July 1, 2005** **Approval Date:** **October 12, 2006**

Funding Sources:

Funding Agency: North Pacific Marine Science Foundation
Funding Title: #57 Captive Northern Fur Seals

Unfunded title: N/A

The Animal Care Committee has examined and approved the use of animals for the above experimental project.

This certificate is valid for one year from the above start or approval date (whichever is later) provided there is no change in the experimental procedures. Annual review is required by the CCAC and some granting agencies.

A copy of this certificate must be displayed in your animal facility.

Office of Research Services and Administration

Table 1. Annual Takes (activities limited to the month of October during the year of capture):

Species	Sex and Age class	Activity	No. of animals taken / year	No. of takes / individual / year	Location
Level A Harassment					
Northern fur seal (*Callorhinus ursinus*)	Pups that appear to be near weaning	Capture via hoop-net, physical restraint, initial gender examination	32	1	St. Paul Island, Bering Sea, AK
		Further physical restraint (of female pups), initial gender and gross health examination	16	1	St. Paul Island, Bering Sea, AK
		Accidental mortality	1	1	St. Paul Island, Bering Sea, AK
		Temporary holding (5-7 days) in enclosure near rookery for health testing (blood samples, eye and oral exams)	8	3	St. Paul Island, Bering Sea, AK
		Fecal loops	8	2	St. Paul Island, Bering Sea, AK
		Marking (bleach)	8	2	St. Paul Island, Bering Sea, AK
		Transportation to Vancouver, Canada to partake in detailed physiological studies	6	1	Vancouver Aquarium, Canada
Level B Harassment only:					
Northern fur	Pups	Disturbance to peripheral animals	100	1	St. Paul Island,

seal (*Callorhinus ursinus*)	Breeding females	during monitoring of site and capture and release of pups	50	1	Bering Sea, AK
	Breeding males		10	1	
	Immature males		25	1	

Table 2. Annual takes during research year.

Species	**Sex and Age class**	**Activity**	**Number of animals taken per year**	**Number of. takes per individual per year**	**Location**
Level A Harassment					
Northern fur seal (*Callorhinus ursinus*)	Female pups (1-2 y)	Physical restraint, blood sampling (first 6 months)	6	12	Vancouver Aquarium, Canada
		Blood sampling under anesthesia	6	12	Vancouver Aquarium, Canada
		Morphological measurements (physical restraint – first 6 months)	6	24	Vancouver Aquarium, Canada
		Morphological measurements (trained)	6	48	Vancouver Aquarium, Canada
		Blubber biopsies (under anesthesia) – takes also included in blood sampling	6	3	Vancouver Aquarium, Canada
		Deuterium oxide injection	6	6	Vancouver Aquarium, Canada
		Ultrasound	6	6	Vancouver Aquarium, Canada

NORTH PACIFIC UNIVERSITIES MARINE MAMMAL RESEARCH CONSORTIUM

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List of Publications and Dissertations Stemming in Part or in Whole from Captive Studies of Steller Sea Lions at the Vancouver Aquarium

PUBLICATIONS:

Hastie, G.D., Rosen, D.A.S., Trites, A.W., in press. The influence of depth on a breath-hold diver: predicting the diving metabolism of Steller sea lions (*Eumetopias jubatus*). Journal of Experimental Marine Biology and Ecology.

Rosen, D.A.S., Winship, A.J., Hoopes, L.A., in press. Thermal and digestive constraints to foraging behavior in marine mammals. Philosophical Transactions, Royal Society of London

Cheneval, O., R. W. Blake, A. W. Trites and K. H. S. Chan. 2007. **T**urning maneuvers in Steller sea lions (*Eumatopias jubatus*). Marine Mammal Science 23:94-109.

Deagle, B.E. and D.J. Tollit. 2007. Quantitative analysis of prey DNA in pinniped faeces: potential to estimate diet composition? Conservation Genetics 8:743-747

Hastie, G.D., Rosen, D.A.S., Trites, A.W., 2007. Oxygen consumption decreases during dives by Steller sea lions (*Eumetopias jubatus*). Marine Mammal Science 23: 272-286.

Rea, L.D., Rosen, D.A.S., Trites, A.W., 2007. Utilization of stored energy reserves during fasting varies by age and season in Steller sea lions. Canadian Journal of Zoology 85: 190-200.

Tollit, D.J., S.G. Heaslip, R.L. Barrick and A.W. Trites. 2007. Impact of diet index selection and the digestion of prey hard remains on determining the diet of the Steller sea lion (*Eumetopias jubatus*). Canadian Journal of Zoology 85:1-15.

Hastie, G.D., Rosen, D.A.S., Trites, A.W., 2006. Studying trained Steller sea lions in the open ocean. In: Trites, A., Atkinson, S., DeMaster, D., Fritz, L., Gelatt, T., Rea, L., Wynne, K. (Eds.), Sea lions of the world, Alaska Sea Grant College Program, University of Alaska Fairbanks, pp. 193-204

Kumagai, S., Rosen, D.A.S., Trites, A.W., 2006. Body mass and composition responses to short-term low energy intake are seasonally dependent in Steller sea lions (*Eumetopias jubatus*). Journal of Comparative Physiology B 176, 589-598.

Richmond, J.P., Jeanniard du Dot, T., Rosen, D.A.S., Zinn, S.A., 2006. Effects of prey composition on the endocrine response to nutrient restriction and re-alimination in Steller sea lions (*Eumetopias jubatus*). In: Symposia of the Comparative Nutrition Society, vol. 6. Keystone, Colorado, pp. 136-141.

Rosen, D.A.S., Tollit, D., Trites, A.W., Winship, A.J., 2006. Potential effects of short-term prey changes on sea lion physiology. In: Trites, A., Atkinson, S., DeMaster, D., Fritz, L., Gelatt, T., Rea, L., Wynne, K. (Eds.), Sea lions of the world, Alaska Sea Grant College Program, University of Alaska Fairbanks, pp. 103-116

Rosen, D.A.S., Winship, A.J., Hoopes, L.A., 2006. Interacting physiological constraints to foraging behavior in marine mammals. In: Symposia of the Comparative Nutrition Society, vol. 6. Keystone, Colorado, pp. 151-156

Tollit, D., Heaslip, S.G., Deagle, B., Iverson, S.J., Joy, R., Rosen, D.A.S., Trites, A.W., 2006. Estimating diet composition in sea lions: what technique to choose? In: Trites, A., Atkinson, S., DeMaster, D., Fritz, L., Gelatt, T., Rea, L., Wynne, K. (Eds.), Sea lions of the world, Alaska Sea Grant College Program, University of Alaska Fairbanks, pp. 293-307

Winship, A.J., Hunter, A.M.J., Rosen, D.A.S., Trites, A.W., 2006. Food consumption of sea lions: data gaps and direction for future research. In: Trites, A., Atkinson, S., DeMaster, D., Fritz, L., Gelatt, T., Rea, L., Wynne, K. (Eds.), Sea lions of the world, Alaska Sea Grant College Program, University of Alaska Fairbanks, pp. 171-191.

Deagle, B.E., D.J. Tollit, S.N. Jarman, M.A. Hindell, A.W. Trites and N.J.Gales. 2005. Molecular scatology as a tool to study diet: analysis of prey DNA in scats from captive Steller sea lions. Molecular Ecology 14: 1831-1842.

Olawale, K.O., R.J. Petrell, D.G. Michelson, A.W. Trites. 2005. The dielectric properties of the cranial skin of five young captive Steller sea lions (*Eumetopias jubatus*) and a similar number of young domestic pigs (*Sus scrofa*) and sheep (*Ovis aries*) between 0.1 and 10 GHz. Physiological Measurement. 26:626-637.

Rosen, D. A. S. and Trites, A. W. 2005. Examining the potential for nutritional stress in young Steller sea lions: physiological effects of prey composition. Journal of Comparative Physiology B 175: 265-273.

Willis, K., and M. Horning. 2005. A novel approach to measuring heat flux in swimming animals. Journal of Experimental Marine Biology and Ecology 315:147-162.

Willis, K., M. Horning, D.A.S. Rosen and A.W. Trites. 2005 Spatial variation of heat flux in Steller sea lions: evidence for consistent avenues of heat exchange along the body trunk. Journal of Experimental Marine Biology and Ecology 315:163-175.

Hoopes, L.A., L.D Rea, D.A.S Rosen, and G.A.J Worthy. 2004. Effects of body condition on resting metabolism in captive and free-ranging Steller sea lions (*Eumetopias jubatus*). Symposia of the Comparative Nutrition Society 2004. No. 5, Pp 79-82.

Hunt, K.E., A.W. Trites, and S.K. Wasser. 2004. Validation of a fecal glucocorticoid assay for Steller sea lions (*Eumetopias jubatus*). Physiology and Behavior 80: 595-601.

Rosen, D.A.S., G.D. Hastie, and A.W. Trites. 2004. Searching for stress: Hematological indicators of nutritional inadequacies in Steller sea lions. Symposia of the Comparative Nutrition Society 2004. No. 5, Pp 145-149.

Rosen, D.A.S. and A.W. Trites. 2004. Satiation and compensation for short-term changes in food quality and availability in young Steller sea lions (*Eumetopias jubatus*). Canadian Journal of Zoology 82:1061-1069.

Tollit, D.J., S.G. Heaslip, T.K. Zeppelin, R. Joy, K.A. Call and A.W. Trites. 2004. A method to improve size estimates of walleye pollock and Atka mackerel consumed by pinnipeds using digestion correction factors applied to bones and otoliths recovered in scats. Fishery Bulletin 102: 509-521.

McPhee, J.M., D.A.S. Rosen, R.D. Andrews, and A.W. Trites. 2003. Predicting metabolic rate from heart rate for juvenile Steller sea lions *Eumetopias jubatus*. Journal of Experimental Biology 206: 1941-1951.

Rosen, D.A.S. and A.W. Trites. 2003. No evidence for bioenergetic interaction between digestion and thermoregulation in Steller sea lions, *Eumetopias jubatus.* Physiological and Biochemical Zoology. 76:899-906.

Tollit, D.J., M. Wong, A.J. Winship, D.A.S. Rosen, and A.W. Trites. 2003. Quantifying errors associated with using prey skeletal structures from fecal samples to determine the diet of Steller's sea lion (*Eumetopias jubatus*). Marine Mammal Science 19:724-744.

Winship, A.J, and A.W. Trites. 2003. Prey consumption by Steller sea lions in Alaska: how much do they require? Fishery Bulletin 101:147-163.

Cottrell, P.E. and A.W. Trites. 2002. Classifying prey hard part structures recovered from fecal remains of captive Steller sea lions (*Eumetopias jubatus*). Marine Mammal Science 18:525-539.

Rosen, D.A.S. and A.W. Trites. 2002. Changes in metabolism in response to fasting and food restriction in the Steller sea lion (*Eumetopias jubatus*). Comparative Biochemistry and Physiology. 132: 389-399.

Rosen, D.A.S. and A.W. Trites. 2002. Cost of transport in Steller sea lions, *Eumetopias jubatus*. Marine Mammal Science 18:513-524.

Rosen, D. A. S. and A. W. Trites. 2002. What is it about food? Examining possible mechanisms with captive Steller sea lions. Pages 45-48 *In* D. DeMaster and S. Atkinson, eds. Steller sea lion decline: is it food II. University of Alaska Sea Grant, AK-SG-02-02, Fairbanks.

Winship, A.J, A.W. Trites, and D.A.S. Rosen. 2002. A bioenergetics model for estimating the food requirements of Steller sea lions (*Eumetopias jubatus*) in Alaska. Marine Ecology Progress Series 229:291-312.

Hirons, A.C., D.M. Schell and D.J. St. Aubin. 2001. Growth rates of vibrissae of harbor seals (*Phoca vitulina*) and Steller sea lions (*Eumetopias jubatus*). Canadian Journal of Zoology 79:1053-1061.

Berman, M., and L. Rea. 2000. The effects of food deprivation on serum lipid concentration and content in Steller sea lions (*Eumetopias jubatus*). *In* C.L.K. Baer (ed.). Proceedings of the Third Comparative Nutrition Society Symposium, No. 3, Pages 13-16. Pacific Grove, California, August 4-9, 2000.

Rea, L.D. and T.R. Nagy 2000. Changes in serum leptin levels during fasting and food limitation in Steller sea lions (*Eumetopias jubatus*). *In* C.L.K. Baer (ed.). Proceedings of the Third Comparative Nutrition Society Symposium, No. 3, Pages 171-175. Pacific Grove, California, August 4-9, 2000.

Rea, L.D., D.A.S. Rosen and A.W. Trites. 2000. Metabolic response to fasting in 6-week-old Steller sea lion pups (*Eumetopias jubatus*). Canadian Journal of Zoology 78:890-894.

Rosen, D.A.S. and A.W. Trites. 2000. Pollock and the decline of Steller sea lions: testing the junk-food hypothesis. Canadian Journal of Zoology 78:1243-1258.

Rosen, D.A.S. and A.W. Trites. 2000. Assessing the role of nutritional stress in the decline of wild populations: a Steller case of scientific sleuthing. *In* C.L.K. Baer (ed.). Proceedings of the Third Comparative Nutrition Society Symposium, No. 3, Pages 182-186. Pacific Grove, California, August 4-9, 2000.

Rosen, D.A.S. and A.W. Trites. 2000. Digestive efficiency and dry-matter digestibility of Steller sea lions fed herring, pollock, salmon and squid. Canadian Journal of Zoology 78: 234-239

Rosen, D.A.S., L. Williams and A.W. Trites. 2000. Effect of ration size and meal frequency on digestive and assimilation efficiency in yearling Steller sea lions, *Eumetopias jubatus*. Aquatic Mammals 26:76-82.

Stelle, L.L., R.W. Blake and A.W. Trites. 2000. Hydrodynamic drag in Steller sea lions (*Eumetopias jubatus*). Journal of Experimental Biology 203:1915-1923.

Rea, L.D., D.A.S. Rosen and A.W. Trites. 1999. Seasonal differences in adaptation to prolonged fasting in juvenile Steller sea lions (*Eumetopias jubatus*).The FASEB Journal (Federation of American Societies of Experimental Biology). Volume 13, Number 5, Page A740. Washington, D.C. April 17-21, 1999.

Rosen, D.A.S. and A.W. Trites. 1999. Metabolic effects of low-energy diet on Steller sea lions, *Eumetopias jubatus*. Physiological Zoology 72:723-731.

Rea, L.D., D.A.S. Rosen and A.W. Trites. 1998. Blood chemistry and body mass changes during fasting in juvenile Steller sea lions (*Eumetopias jubatus*). Proceedings of the Comparative Nutrition Society, Number 2, pg. 174 - 178. Banff, Alberta, Canada August 14-19, 1998.

Rosen, D.A.S. and A.W. Trites. 1998. Changes in metabolism in response to varying energy intake in a marine mammal, the Steller sea lion. Proceedings of the Comparative Nutrition Society, Banff Alberta, August 1998, pp. 182-187.

Rosen, D.A.S. and A.W. Trites. 1997. Heat increment of feeding in Steller sea lions, *Eumetopias jubatus*. Comparative Biochemistry and Physiology 118A: 877-881.

DISSERTATIONS:

Cheneval, O. 2005. Biomechanics of turning manoeuvres in Steller sea lions (*Eumetopias jubatus*). Masters thesis. University of British Columbia, Vancouver BC. 64 pages.

Kumagai, S. 2004. Seasonal differences in physiology of captive Steller sea lions (*Eumetopias jubatus*) in response to short-term low energy intake. Masters thesis. University of British Columbia, Vancouver BC. 95 pages.

Daniel, R.G. 2003. The timing of moulting in wild and captive Steller sea lions (*Eumetopias jubatus*). Masters thesis. University of British Columbia, Vancouver BC. 64 pages.

Azana, C.D.P. 2002. Seasonal variation in nutrient composition of Alaskan walleye pollock (*Theragra* chalcogramma) and its effect on the nutritional status of Steller sea lions (*Eumetopias jubatus*). Masters thesis. University of British Columbia, Vancouver BC. 104 pages.

McPhee, C.P. 2001. Heart rate as a monitor for metabolic rate in captive juvenile Steller sea lions (*Eumetopias jubatus*). Masters thesis. University of British Columbia, Vancouver BC. 86 pages.

Winship, A.J. 2000. Growth and bioenergetic models for Steller sea lions (*Eumetopias jubatus*) in Alaska. Masters thesis. University of British Columbia, Vancouver BC. 160 pages.

Stelle, Lei Lani. 1997. Drag and energetics of swimming in Steller sea lions (*Eumetopias jubatus*). Masters thesis. University of British Columbia, Vancouver BC.