I. TITLE OF APPLICATION: Application for a Permit for Scientific Research under the Marine Mammal Protection Act and the Fur Seal Act.

II. DATE OF APPLICATION: January 15, 2007

III. APPLICANT AND PERSONNEL:

A. <u>Contact Information for Applicant and Personnel:</u>

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B. <u>Qualifications and Experience</u>

Andrew Trites, Ph.D. directs the research of the North Pacific Universities Marine Mammal Research Consortium. He is a Professor and Director of the Marine Mammal Research Unit at the University of British Columbia, and has extensive experience studying northern fur seals and Steller sea lions. A copy of his CV is attached

David Rosen, Ph.D. is a research scientist at the Marine Mammal Research Unit, and is in charge of the captive Steller sea lion research

program. His graduate work was on the behavioral ecology and ecophysiology of pinnipeds. He has worked with captive pinnipeds for over 20 years. A copy of his CV is attached.

Drs. Trites and Rosen have developed a world class facility at the Vancouver Aquarium for studying pinnipeds in captivity, and have a strong record of producing and publishing timely research findings. Dr. Trites will oversee the northern fur seal research program as the Director of the Marine Mammal Research Unit. Dr. Rosen will be responsible for carrying out the individual proposed experiments, either directly or via supervised technicians and graduate students.

Dr. Chris Harvey-Clark is the Director of the Animal Care Center at the University of British Columbia. He has extensive research and veterinary experience working with captive and wild animals. Dr. Harvey-Clark will be primarily responsible for the health and safety of the animals from the time of capture until they are brought to the Vancouver Aquarium, including the initial health/suitability assessments. Dr. Harvey-Clark will also assist the staff veterinarian at the Aquarium with the continued monitoring of the animals while in captivity.

IV. PROPOSAL

A. Summary

The proposed project involves conducting physiological studies on young, captive northern fur seals (*Callorhinus ursinus*) that are critical to understanding the reasons for their population decline in the wild. We will test the hypothesis that changes in their food supply and/or environmental conditions are inducing a state of nutritional stress that is ultimately leading to changes in survival and/or reproductive success. A maximum of 32 northern fur seal pups approaching weaning age will be directly taken (disturbed). This includes up to eight female pups that will be temporarily removed from the rookery to a holding facility on the island for up to 7 days. Six of these pups will be removed from the island and transported to the Marine Mammal Species At Risk Research Laboratory in Vancouver, Canada. In the process of the captures, up to 185 northern fur seals may be temporarily, indirectly disturbed, although every effort will be made to minimize the impact of these actions. The pups that are removed from the island will participate in a series of long-term studies that test observed and/or hypothesized relationships between diet, environmental conditions, and population trends. The research will concentrate on identifying indicators of food limitations (which can also be used as a field measurement), and determining physiological responses and adaptations to nutritional stress imposed by manipulating their diet (quality and quantity), in combination with changes in their environment (water temperature). Data will also be collected to parameterize a bioenergetic model needed to determine the food requirements and physiological basis of foraging behavior of fur seals. The fur seal pups will also be used to develop and

validate research techniques that can be applied to their wild counterparts (diet analyses, telemetry equipment). These studies can not be undertaken with wild fur seals, and can only be accomplished with trained animals in a controlled setting. We are requesting this permit for 5 years.

B. Introduction:

1a. Target species:

Northern fur seal (*Callorhinus ursinus*). Permission is requested to temporarily hold 8 female northern fur seal pups for up to 7 days in October 2007, and to permanently take 6 of these pups for permanent holding in Vancouver, Canada.

1b. Non-target species:

No other species (pinniped or seabird) will be taken incidentally during the course of our activities.

1c. Status of Affected Stock(s):

The Pribilof Island/Eastern Pacific stock was listed as Depleted under the Marine Mammal Protection Act on 17 June 1988 (Federal Register 53 FR 17888). The species is not listed under CITES, but is listed as Vulnerable (VU A1b) under the IUCN Red List.

2. Background/Literature Review

The northern fur seal population on the Pribilof Islands (St. Paul and St. George Island), in the central Bering Sea, is the largest U.S. population and accounts for about 56% of the world's population. The Pribilof Island rookeries are the principal breeding areas for this species (NMFS, 1993; Gentry, 1998; York et al., 2000). In 1988 it was determined that northern fur seals were below their optimum sustainable population level and were listed as "Depleted" under the Marine Mammal Protection Act.

The 2004 estimate of numbers of pups born on St. Paul Island was 122,825 (SE=1,290), which was 15.7% less than the estimate in 2002, and 22.6% less than the estimate in 2000 (NMFS, 2004). The 2004 pup estimate for St. George Island was 16,876 (SE=239), which was 4.1% less than the estimate in 2002, and 16.4% less than the estimate in 2000. The total number of pups born on the Pribilof Islands reflects a total population estimate of approximately 629,000 animals of all ages. Estimated numbers of pups born declined at 6.2% per year (SE = 0.78%, P = 0.01) on St. Paul Island, and at 4.5% per year (SE = 0.45%, P = 0.01) on St. George Island, from the estimated numbers in 1998. Estimated numbers of pups on the two islands, as a whole, has declined at 6.0% per year (SE = 0.59%, P = 0.01) since 1998 (NMFS, 2004b). The 2004 pup production estimate on St. Paul Island is comparable with the level observed in 1918, while the St. George pup production estimate is below the level observed in 1916 (Angliss and Lodge, 2003; NMFS, 2004a).

There are several environmental and anthropogenic factors that may affect population trends of northern fur seals. These include, but are not limited to, large-scale oceanographic climate change, fishing vessel disturbance, shift in prey species distribution and abundance, competition with fisheries, interactions with commercial fisheries, and subsistence harvesting (Angliss et al., 2001). Anthropogenic impacts may affect the population both directly (e.g. physical damage, disturbance) and indirectly (e.g. association with fishing activity); however there is little direct evidence (Richardson et al., 1995; Gentry, et al., 1998; Gisiner, 1998).

This permit proposes permanently removing 6 pups from the wild to form the core of a captive northern fur seal research colony. The aim of this research program is to directly address questions that can lead to the recovery of the species. Removing animals to a laboratory setting allows researchers to conduct detailed studies in controlled environments that would be impossible to achieve in the wild. The aim of the research is not to duplicate the conditions the animals experience in the wild (even if this were known or possible). Rather, the overall research approach is to control the animal's environment as much as possible, and manipulate key aspects (e.g., diet, temperature) in a controlled fashion to determine their potential impact on their wild counterparts. Empirical research in the laboratory is critical to testing the correlational relationships observed (or hypothesized to occur) in the wild. This approach is particularly suited for questions of physiological function, nutrition, and bioenergetics. The proposed research is largely guided by the recommendations of the National Marine Fisheries Service Final Conservation Plan for Northern Fur Seals (NMFS, 1993). This includes work on the possible role of nutritional stress, and the impact of biotic (fish type and distribution, disease) and abiotic changes (climate change) on northern fur seal populations.

The causes for the continued population decline and lack of recovery of northern fur seals are unknown. The apparent synchrony between the unexplained decline of northern fur seals and other North Pacific marine mammal and seabird species (DeMaster et al., 2006), coupled with apparent changes in North Pacific fish populations and oceanography (Benson and Trites, 2002, Trites et al., 2006), has led to the hypothesis that nutritional stress may be a contributing factor to the population declines of pinnipeds in the North Pacific (Trites and Donnelly, 2003). This nutritional or energetic imbalance may be induced by changes in the distribution or abundance of prey, by either decreasing the energetic value of dominant prey or changing the costs of foraging or other activities. Diet studies indicate that fur seals using the Pribilof Islands primarily consume pollock and squid (Sinclair et al., 1994). Given the generally low caloric densities of these prey species, it would not take a great change in either prey intake or energetic expenditures to place the

animals in energetic imbalance. This likelihood is increased by the potential shifts in fishing efforts away from Steller sea lion habitat towards fur seal foraging grounds. Given the highly specific home ranges of these animals, localized depletion may become a significant factor (Loughlin et al., 1987)

A great deal of research has been completed on the nutrition and physiology of otariids (primarily on Steller sea lions; see electronic database at www.alaskasealife.org/New/research/literature-database.php). However, this same research has shown that their physiological responses are highly species-specific. Therefore, the research we propose to conduct can only be performed on northern fur seals to be relevant to the recovery of that species. Past research with captive northern fur seals is minimal; only 3 of the publications since 1999 in the AFSC Fur Seal Bibliography Database are from work with captive animals, and most of the prior work has been on husbandry issues. Knowledge we have gained in specifically studying northern fur seals in captivity will be used to refine and optimize our experimental designs to obtain results in a timely and efficient manner.

3. Hypothesis/Objectives and Justification

The causes for the continued population decline and lack of recovery of northern fur seals are unknown. The apparent synchrony between the unexplained decline of northern fur seals and other North Pacific marine mammal and seabird species (DeMaster et al., 2006), coupled with apparent changes in North Pacific fish populations and oceanography (Benson and Trites, 2002; Trites et al., 2006), has led to the hypothesis that nutritional stress may be a contributing factor to the population declines of pinnipeds in the North Pacific (Trites and Donnelly, 2003). We propose to conduct physiological studies on young, captive northern fur seals that are critical to understanding the reasons for their population decline in the wild.

We will test the hypothesis that changes in their food supply (type and/or distribution) and/or environmental conditions (i.e., water temperature) are inducing a state of nutritional stress that is ultimately leading to changes in survival and/or reproductive success

The fur seals will participate in a series of long-term studies concentrating on identifying indicators of food limitations, and determining physiological responses and adaptations to nutritional stress. Data will also be collected to parameterize a bioenergetic model needed to determine the food requirements of fur seals. The fur seal pups will also be used to develop and validate research techniques that can be applied to their wild counterparts (diet analyses, telemetry equipment). These studies can not

be undertaken with wild fur seals, and can only be accomplished with trained animals in a controlled setting.

Specific research objectives:

a. Obtain baseline measures of growth and resting and daily metabolism in young northern fur seals to enhance predictive bioenergetic models.
b. Determine the fasting capabilities of young fur seals, and the interaction between fasting and thermal demands.
c. Establish blood biochemistry and hematology parameters that can be used as bioindicators of nutritional stress in northern fur seals.
d. Determine the pattern of tissue catabolism during periods of under-nutrition.
e. Determine the effect of dietary changes on reproductive hormones.
f. Estimate the maximum food intake levels of young northern fur seals and their ability to alter intake to compensate for changes in food quality and availability.
g. Determine the species-specific calibration coefficients (enrichment values) needed to determine diet from fatty acid signature analysis.
h. Quantify digestion and recovery correction factors required to accurately describe diet from hard fecal remains (scat analysis).
i. Determine the effectiveness of using stable isotope and fatty acid signature analyses to determine diet in wild fur seals.

Details on the methodology involved in each of these studies can be found in **Section 2bi** and in the attached funding proposal (*Physiological Studies of Captive Northern Fur Seals*).

Rational for these research projects:

To determine when and if northern fur seals are nutritionally stressed, it is necessary to quantify their energy intake (diet), their energy and nutritional requirements, and their responses to nutritional and energetic imbalance. These data can be incorporated into dynamic bioenergetic models that can be used to predict the physiological and behavioral response of fur seals under differing nutritional and environmental conditions.

Quantifying food intake requires a means to accurately describe the diet of northern fur seals in the wild. The traditional practice of direct analysis of stomach contents has been generally replaced by analysis of fecal hard parts, which has its own inherent limitations and biases (Tollit et al., 2003). More recently a number of techniques have been developed to accomplish this task — fatty acid and stable isotope signature analyses, and genetic tissue identification (Tollit et al., 2006). Only recently have these techniques been subject to extensive testing and quantification of inherent biases. It has become obvious that most of these techniques

require species-specific calibration data to accurately reconstruct food intake. For example, failure to account for differential recovery and digestion rates of fish otoliths and squid beaks will lead to large biases in diet reconstruction for species consuming both types of prey (Tollit et al., 1997). Also, species-specific calibration coefficients for fatty acid signature analysis have been shown to be necessary to accurately describe diets (Budge et al., 2006).

Previous bioenergetic models of pinnipeds have indicated several key variables of energy requirements that can be best parameterized through longitudinal studies of captive animals (e.g., Winship et al., 2002). Resting metabolic rate is a key physiological and comparative measure that forms the backbone of an animals' energy budget. It is related to both minimal energy requirements and maximum sustained energy expenditures (McNab, 1999). It varies both developmentally and on a seasonal basis. Daily metabolic rates are another measure of an animal's bioenergetic capacity and a more direct measure of energy requirements (Nagy, 1987). Developmental and seasonal changes in resting metabolism have been measured in only a few pinnipeds, while fewer measures exist for daily metabolic rates (Hunter, 2005). Physical growth is another key parameter as it has critical long-term effects on survival and reproductive success. The developmental and seasonal patterns of growth (including patterns of core versus lipid growth) are poorly documented in most pinnipeds.

The aforementioned parameters will contribute to the development of a static bioenergetic model that can describe the requirements for a northern fur seal to remain nutritionally and energetically balanced under certain conditions. However, to evaluate the potential impact of changes in the environment on young fur seals (i.e., create a dynamic, predictive model), it is important to understand key aspects of their bioenergetics, particularly in terms of energy partitioning under conditions when food intake is insufficient to meet normal energy and nutritional requirements (Mangel and Clark, 1988). Pinnipeds have a variety of means to adjust to unpredictable changes in food intake (such as those that have been hypothesized in the nutritional stress hypothesis) (Costa et al., 1991; Kumagai et al., 2006). However, the actual response of an individual depends on a number of factors including species, age class, reproductive status, time of year, environmental conditions (temperature), and current health status. Therefore, controlled experiments are required to determine the likely physiological effects of restricted food intake on northern fur seals, taking as many of these variables into account as feasible. For example, traditional fasting physiology theory suggests that under conditions of restricted energy intake, pinnipeds should primarily catabolize their subcutaneous blubber layer to provide additional energy (Cherel et al., 1992). However, this layer must also be conserved as a thermoregulatory defense. While core tissues can provide energy, the sacrifice of key tissues will lead to long-term physiological and anatomical

problems, and eventually death (Øritsland & Markussen, 1992; Rosen et al., in press). Previous research on young Steller sea lions suggests that animals lose a surprisingly high proportion (40-50%) of core tissues during periods of energy restriction and body mass loss (Rosen and Trites, 2005; Kumagai et al., 2006). It is important to understand the effects of energy restriction on body composition, as it has both drastic short-term (thermoregulatory capacity, survival) and long-term effects (e.g., growth, size at maturity, reproductive success) (Rosen et al., in press).

The high rate of core tissue loss exhibited by Steller sea lions during periods of mass loss may be a result of their relatively thin blubber layer compared to phocid seals. Given the climate of the North Pacific and Bering Sea, pinnipeds would be likely to primarily conserve blubber for its thermoregulatory benefit. This could be particularly true for young northern fur seals with their greater surface area to body mass ratios. In these animals, even slight changes in their lipid reserves may have major consequences to their overall energy budget through increased thermoregulatory costs (Trites, 1990; Donohue et al., 2000). Animals can often limit their mass loss by decreasing their metabolism, a physiological response known as metabolic depression (Guppy and Withers, 1999). Although the thermoregulatory capacity of northern fur seals has been previously studied (Ohata and Miller, 1977; Donohue et al., 2000), their ability to respond to simultaneous energetic challenges (thermal and nutritional stress) is unknown. Metabolic depression can interact with thermoregulatory capacity, as the individual is not producing as much heat to maintain core body temperatures. The way an animal balances these factors affects its activity, foraging decisions, and ultimately survival (Rosen et al., in press). Therefore it is important to investigate the interaction between body composition changes and thermoregulatory capabilities during periods of restricted energy intake.

Alternately, an animal subject to decreased prey quality or availability may increase food intake when sufficient prey is available. However, the digestive system represents a physiological limit to such compensation (Weiner, 1992). This is particularly true for young animals that generally have higher relative energy requirements, smaller digestive systems, and decreased foraging (diving) capabilities. Quantifying the digestive capacity of captive Steller sea lions has helped to delineate ecological scenarios where increasing food intake is not a physiologically realistic strategy (Rosen and Trites, 2004). Similar measures of northern fur seals are critical to understanding the physiological attributes that may lead to nutritional stress.

From a practical viewpoint, it is also critical to determine whether nutritional stress is apparent in different segments of the wild fur seal population at different times of the year. Analysis of blood samples has proven a valuable tool for wildlife managers for a number of mammals

(Harder and Kirkpatrick, 1994). Unfortunately, changes in blood profiles are species-specific and depend partly on the duration and severity of nutritional stress (Rosen et al., 2004). We propose to develop simple physiological indicators of nutritional stress. This includes tracking changes in blood biochemistry and hematology, and body composition indices during experimental periods of restricted food intake. Natural variation in these parameters is also critical to calculating approximate sample sizes required to detect these differences between populations. These results can then be applied in the future to samples obtained from wild northern fur seals.

While much physiological research can be undertaken on animals in the wild, they suffer in their general restriction to correlational data, the inability to control external variables, and the difficulties in conducting long-term longitudinal studies. Research with animals in the laboratory presents the advantages of conducting long-term, longitudinal studies using precise manipulations of select variables under controlled conditions. Studies with captive animals can not (and should not) mirror the complexity of the natural environment, but their innate simplicity facilitates the clear testing of specific hypotheses (often testing cause and effect relationships formulated from observational data in the field) and the precise quantification of specific variables. While care must be taken in applying these results within the context of their wild counterparts, interpreting experimental data beyond the specifics of any experiment are a central process of the modern scientific method. Research programs with dedicated populations of captive marine mammals have been instrumental in scientific breakthroughs that would be impossible with animals in the wild.

Choice of species and number of animals:

In the last 30 years, a substantial amount of scientific work has been competed using captive pinnipeds. The majority of species held for research purposes have been phocid seals (“true” seals), which are both anatomically, physiologically, and behaviorally different from otariids (fur seals and sea lions). In the past decade, the value of conservation-directed work with Steller sea lions has become evident through work at several research facilities (see electronic database list of publications at www.alaskasealife.org/New/research/literature-database.php). However, this same research has shown that physiological responses are highly species-specific. Therefore, research needs to be conducted specifically on northern fur seals to be relevant to the recovery of that species. Past research with captive northern fur seals is minimal, and mainly deals with husbandry (see Scott et al., 2006).

In scientific research, it is mathematically better to have larger sample sizes in order to minimize type II error (beta; the chance of missing a

significant difference/effect). Smaller sample sizes will result in a lower power of the statistical test to correctly reject the null hypothesis.

However, large sample sizes are not always feasible, particularly in certain types of research (e.g., studies with large captive vertebrates). In our case, we must balance the statistical requirement for more animals with the logistical constraints of humanely housing and working with an appropriate number of individuals and the ecological impact of removing more individuals from a depleted population.

Scientists can mitigate the statistical effects of small sample sizes through several avenues which all relate to study design. One of these protocols is to use the strength of repeated measures designs to mitigate the effects of inter-individual variation. For example, a sample size of 6 animals taking part in a two-diet manipulation (one way ANOVA) gives an *a priori* statistical power value of 0.27 (low). However, if a complete cross-over design is employed, the statistical power value increases to 0.76 (medium). Several other measures will be integrated into the proposed study designs to increase the effective power of the manipulations. These include:

- High innate consistency between individuals (gender, age, holding conditions, health),
- Simplify and magnify experimental manipulations (i.e., minimize experimental factors),
- Minimize non-experimental manipulations (e.g., consistency in environmental conditions, non-crossover between experiments).

We feel that a group of 6 northern fur seals represent a reasonable compromise that allows us to perform valid scientific experiments with a healthy, well-cared for group of captive fur seals.

C. Methods:

1. Duration of the Project and Locations of Taking:

Initial takes and holding/evaluation are anticipated to be for 5-7 days in October 2007 on St. Paul Island (57° N, 170°W) in the Bering Sea, Alaska. This time of year corresponds to when fur seals are weaning. With the assistance of experienced NMFS researchers, 8 female pups that are approaching weaning will be captured from the fringes of one rookery on St. Paul Island (possibly Zapadni Reef, Kitovi, Vostochni, English Bay or Reef rookery). Although the portion of the study (Take) covered by the permit will only occur over 1 field season, we are requesting a 5-year permit since the logistical coordination required for both the field site and the holding/research facilities may mean that the

captures will not occur in the first year of the permit. However, actual takes will only occur in one year.

The animals will be held within a specially-built enclosure near their rookery of birth, where initial health and suitability testing will take place. Animals will initially be fed in water-filled feeding troughs (to minimize imprinting), using previously frozen herring and capelin. Food will be transported frozen, and kept in a local freezer until required on a daily basis. Over the next 5-7 days, the group will be decreased to 6 pups, based primarily on health parameters (physical examinations, blood parameters that can be analyzed on site, fecal parasite loads) and secondarily on behavioral characteristics (aggression, level of activity, alertness), and lastly on their inclination to consume dead fish. The short period of holding and the young ages of the pups mean that the two animals not selected for the research program will continue their normal life history when returned to the rookery where they were obtained. The remaining 6 animals will be transported by air to Vancouver, Canada, and will be housed in the Marine Mammal Species-At-Risk Research Laboratory at the Vancouver Aquarium after a 30-d quarantine period at the Vancouver Aquarium Rehabilitation Centre.

Funding to undertake long-term research studies with these animals is anticipated for a minimum of 4 years. The animals will be predominantly held in a non-public research area at the Vancouver Aquarium, and will be intermittently on public display for logistical and education purposes. These highly trained individuals will be permanently housed at the MMSAR Laboratory for participation in research critical to species recovery efforts, starting with the studies listed within. It is unfeasible to reintroduce these animals back into the wild.

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

a. Estimated Takes:

The proposed research involves both **Level A** and **Level B** harassment of northern fur seals. No other takes of vertebrate species are anticipated. All takes of northern fur seals by incidental disturbance and direct takes are summarized according to number, type and age in **Table 1**. Additional information is provided in **Table 2** on annual Takes during the subsequent research program in Vancouver.

The greatest portion of the estimated number of takes consists of incidental disturbance, or unintended harassment. Estimates of both direct takes and incidental disturbance represent the maximum levels; actual numbers incidentally disturbed are likely to be much lower. Table 1 lists the number of animals that might be disturbed assuming the need to capture the maximum number of pups (32) to obtain the

required initial testing group of 8 females. This assumes that only half of the pups captured will be female and that only half of these will be candidates for captivity. Although we are requesting a 5-year Permit to account for the logistical difficulties of carrying out the pup captures, the actual capture events (and therefore direct and indirect takes) will only occur in one year.

We hope to decrease disturbance by not taking the maximum number of pups and by using capture techniques that minimize harassment of peripheral animals for any given capture. Harassment or incidental disturbance refers to a physical response by an animal in recognition of activity on the breeding site, ranging from adjusting body posture to moving away from territory. Incidental disturbance of northern fur seals will be to both male and female pups, as well as mature animals that may still be present on shore.

Direct takes refer to the number of animals that will be handled and released by researchers. We propose to temporarily hold 8 female pups approaching the age of weaning in October 2007. To obtain 8 suitable candidates, up to 32 pups may be captured for initial gender examinations and up to 16 for gross physical examinations. These inspections will be quick (<20 minutes for gender identification, <1 hr for initial health examinations), and should have no long-term effects on the behavior or survival of the animals returned to the rookery. The 8 female pups that pass this initial selection process will each be subject to 3 detailed health examinations that will take <90 minutes each. Six of these animals will be permanently transported to Vancouver, Canada, to take part in research studies.

Pups will be removed from the rookery at the time when weaning naturally occurs. These young animals are the portion of the population of scientific interest, as their higher relative metabolic demands and lower diving and foraging capabilities make them more susceptible to nutritional stress. Additionally, past experience has shown that it is easier to train pinnipeds prior to nutritional independence. Since the planned experiments require trained, co-operative, calm animals, it is preferable to capture the animals after they are old enough to survive without maternal nutritional input, but prior to complete independence.

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
		Transportation to Vancouver, Canada to partake in detailed physiological studies	6	1	Vancouver Aquarium, Canada
Level B Harassment only:					
Northern fur seal (*Callorhinus*	Pups	Disturbance to peripheral animals during monitoring of site and capture and release of pups	100	1	St. Paul Island, Bering Sea, AK
	Breeding females		50	1	

ursinus)	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)	8	48	Vancouver Aquarium, Canada
		Blubber biopsies (under anesthesia) – takes also included in blood sampling	6	3	Vancouver Aquarium, Canada

b. Research and/or Enhancement Methods

i. Capture and Restraint:

We plan to hold 8 healthy female pups for 5-7 days, in an enclosure adjacent to the rookery, or near the NMFS research facilities. At the end of this phase, 6 of the fur seals will be transported to Vancouver, and the other 2 will be released back onto the rookery. In order to obtain the 8 initial subjects, we have allowed for a total of 32 pups to be captured and restrained for varying periods of time, depending on the type of exam required. We plan on performing all of the initial captures during 2 events over 2 days. The initial determination for gender (max take 32 animals) will require restraint for only up to 20 minutes. Gross physical exams of potential female pups (max take 16 animals) will take up to 60 minutes. Detailed examination of the remaining 8 females (blood sampling, oral and eye examinations, fecal samples) will take up to 90 minutes (and will be repeated up to 3 times before final selection).

Prior to captures, observers will determine which pups are old enough to potentially wean (based on size), but are still nursing. Location and naturally occurring marks will be used to identify individuals. Observers will also determine when females are away from their pups before capture attempts. Captures will begin by slowly crawling towards the subject while making maximal use of the local topography (e.g., boulders) to allow close approach without being detected. Such an approach minimizes disturbance to any surrounding fur seals while getting within capture range of a potential subject, and at the same time provides the opportunity to observe and confirm the subjects. Pups will be captured with a reinforced hoop-net (ca. 0.8 m diameter) and carried a short distance away (e.g., 10-20 m; out of sight from other animals). Pups that are situated more towards the periphery of the rookery will be preferentially chosen to minimize the impact of the capture on other animals. However, care will also be taken to avoid individuals that are ‘isolated’ from the rookery, which might indicate compromised individuals.

We will use physical restraint within the hoop net by trained personnel for the initial gender exam. Although the ‘take’ time for these are estimated at a maximum of 20 min actual physical restraint time will be a maximum of 5 min (the remainder being transport and capture time). Male pups will be immediately returned to the rookery where they were obtained. For longer restraints we will employ a standard restraining board (see Antonelis, 1992) utilizing a neoprene “H” harness that serves to hold the seal’s fore-flippers in tight against their body. Once restrained, an initial physical exam will be performed (cuts, eye or mouth problems, outward signs of disease or injury). If deemed inappropriate based on these initial examinations, the animal will be returned as quickly as possible, with minimal disturbance, to the area of

the rookery where it was removed. Suitable animals will then be subject to a more detailed physical examination. A blood sample (10 mL from caudal gluteal) and fecal loop for parasite ova analysis will be obtained. An identification mark will be shaved into the fur on its hip using hair clippers, and the animal will be transferred to the holding area. Blood samples will be analyzed immediately on-site for CBC and key blood biochemistry (e.g., creatinine, alkaline phosphatase, gamma GT, various electrolyte levels). Parasite loads will also be determined on-site.

Animals that pass the initial health selection process will be held ~40 meters from the beach in a specially built enclosure. A 12 by 10 foot area will be enclosed by a temporary fence, with the option of dividing the enclosure into two sections for either animal separations or to provide an enclosed work area where the planned subsequent health examinations can take place. The enclosure will be manufactured from materials that are free from sharp edges, and high enough to prevent escape.

A small, shallow pool of water will also be provided for thermal relief and play. The pool will be constructed by digging a depression in the ground, and overlaying with a waterproof tarp, providing a more natural water source that does not present a physical hazard to the pups. During sessions when the 8 animals are being fed, this pool will be used as a food trough. Previously frozen herring and capelin will be left in the cleaned water trough for short periods (<60 min, depending on weather conditions). Food will be transported to the island frozen, and kept in a local freezer until required on a daily basis. The enclosure will be cleaned once per day using a shovel to remove waste and applying a new layer of cover. Water pools will be cleaned at least twice per day with disinfectant and refilled using clean sea water.

The 6 animals that make the final selection will be transported to Vancouver, B.C. by chartered airline, accompanied by a veterinarian and experienced support staff. They will be transported in large canine kennels. The animals will be kept in close proximity to each other, as they are likely to have become socialized during their time in the beach enclosure. However, only one pup will be held in each kennel to avoid inadvertent injuries. Although fur seals are not susceptible to hyperthermia in transit at normal fall temperatures, they will be closely monitored in transit by vet staff. Upon arrival in Vancouver, the animals will be held at the Vancouver Aquarium Rehabilitation Centre for more detailed health assessments (including tests for viral contagions). Finally, the animals will be transferred to the Marine Mammal Species-At-Risk Laboratory, which is in the off-display area of the Vancouver Aquarium. This area was especially designed for research of captive pinnipeds, and has been used extensively for Steller sea lion studies. It has 4 pools (minimum 10 foot depth) with ample haul out space, as well

as numerous dry holding areas (detailed in **Section 3g**). Although several species of marine mammals can be simultaneously held in different parts of the research area, care will be taken with mixing the northern fur seals only with appropriate individuals.

Methodologies for Proposed Research:
The following describes the research to be completed with the fur seals upon arrival in Vancouver. All behaviors are done voluntarily with trained animals (using positive reinforcement training methods only) unless otherwise indicated. All biological sampling is performed under the supervision of a qualified veterinarian. Blood samples will initially be taken under physical restraint, and later under anesthesia once the animals are sufficiently trained (max 6 months). All biopsies are completed under anesthesia. Weekly morphological measurements will be completed under physical restraint until trained (max 6 months), and then on a voluntary basis (see Table 2).

a. Obtain baseline measures of growth, and resting and daily metabolism
Previous bioenergetic models of pinnipeds have indicated several key variables of energy requirements that can be best parameterized through longitudinal studies of captive animals (e.g., Winship et al., 2002). These include growth rates and efficiency, resting metabolism, and daily metabolic rate. Body mass and food intake will be measured daily, and animals will be subject to a set of morphological measurements twice a week (using a rope and ruler). These include total body length, body girth at 3 locations along the trunk, and dorsal and lateral blubber depth at these same locations. Blubber depth is determined with a Sonosite 180 machine. Body composition will also be determined every 2 months using deuterium dilution techniques (Reilly and Fedak, 1990), and conversions from Arnould et al. (1996). The proximate composition and total energy content of all prey items are also recorded.

Resting metabolism is measured for 90 minutes every 6 weeks using indirect respirometry in the dry metabolic chamber (~1,050 L), and daily metabolic rate is measured over 24 hr in the large metabolic tank every 6 weeks. Measurements of standard metabolic rate are completed under conditions for non-mature animals - postabsorptive, quiescent but awake, non-pregnant, and within their assumed thermal neutral zone (Kleiber, 1975). The animals enter a sealed chamber, and air is drawn through at a constant rate (150-200 L min^{-1}) using a 500H Flow Generator & Controller (Sable Systems, Salt Lake City, UT) sufficient to prevent extreme change in gas concentrations (O_2>19.5%, CO_2<1.9%). Air circulation is ensured by a small fan, and sea lion

behavior is monitored via a video camera. Temperature within the chambers is measured remotely.

Oxygen and carbon dioxide concentrations within a desiccated subsample of expired air are determined by a FC-1B Oxygen Analyzer, a CA-1B Carbon Dioxide Analyzer, (Sable Systems Henderson, NV). Expired air current is continuously sub-sampled, and a Sable Data Acquisition System takes an average concentration from 100 sub-samples every second. Oxygen consumption rates are converted to energy equivalents using appropriate respiratory quotients.

b. Fasting capabilities and the impact of concurrent thermal demands

Previous studies with young Steller sea lions have shown that these animals display rapid, substantial metabolic depression during short periods of fasting. This is presumably a defensive physiological adaptation to maternal foraging/attendance patterns. However, metabolic depression limits the capacity for thermoregulatory responses. In Steller sea lions, the responses to concurrent fasting and thermal challenges were dependent on age, initial body condition, and initial rate of mass loss (Rosen, unpubl. data). This response is likely also affected by seasonal changes in energetic priorities. The nature of the response is a significant, complex interaction between climate, fur seal fasting capabilities, and individual foraging success.

We propose conducting experiments on the metabolic response of fur seals during a 48-hour fast during winter and summer months. These experiments will provide data on the comparative ability of young northern fur seals to thermoregulate in cold water before and during a period of nutritional stress. The results will quantify the energetic costs of short periods of nutritional stress on young fur seals.

Resting metabolism will be measured at 0, 24, and 48 hr into the fasting period and 24 hr post-refeeding. Immediately following each measure of resting metabolism will be a measure of metabolism during a thermal challenge using a specially designed metabolic chamber that can be partly filled with water. Water from a large reservoir (set temperature 2°C) is continuously pumped into the chamber to maintain a constant thermal gradient and to cover the trunk of the animal. Water temperatures (inflow/outflow) and depth are constantly monitored. Metabolism is measured via gas respirometry (oxygen consumption rates) using Sable System (Henderson, NV) analysers and samplers. Rectal body temperature will also be measured during and after the trials, and body condition will be determined at the start and end of each experiment using deuterium dilution techniques. Blood samples

will also be obtained for clinical and experimental analyses (see next Methods).

c. Blood biochemistry and hematology as bio-indicators of nutritional stress and

d. Pattern of tissue catabolism during periods of under-nutrition and

e. Determine the effect of dietary changes on reproductive hormones.

Studies with captive Steller sea lions have indicated that, contrary to the previously-established pinniped model, these animals utilize a large proportion of core tissues during periods of energy restriction and body mass loss (Rosen and Trites, 2005; Kumagai et al., 2006). While this might be a species-specific response, it is more likely a general Otariid strategy resulting from their relatively limited lipid reserves (compared to phocid seals). During these experiments, we also identified a subset of blood parameters that appear to be viable biomarkers of nutritional stress (Rosen et al., 2004). The physiological response of northern fur seals to such short-term food restrictions is unclear, particularly given that they possess a thinner blubber layer than Steller sea lions but additional thermoregulatory capacity from their pelage.

We propose to conduct experiments to determine the effects of food restriction on body lipid stores and other aspects of tissue catabolism and blood profiles in northern fur seals. The food intake of the animals will be initially monitored over a 28-d control period. The animals will then be held on submaintenance diets, at levels designed to lose 10-15% of their body mass over 42 days. This is followed by a 28-d recovery period where the animals receive the same quantity of food as they consumed during the control period. This phase of the experiment is designed to determine the scope and mechanisms of compensatory growth. During a final 28-d ‘ad lib’ phase, the animals will be allowed to consume as much food as their training protocol permits. Each fur seal will repeat the experiment for each of three proposed diet types that differ in their composition. The dietary manipulations will include high lipid, low protein (e.g., herring), a single species moderate protein and lipid (e.g., pilchard), and a mixed species that combines into an alternate moderate protein diet (e.g., herring and squid).

Blood samples (~25 mL total) will be obtained and body composition will be determined through deuterium dilution every 2 weeks during the entire experiment. Serum and dose samples for the deuterium dilution technique are analyzed by Metabolic Solutions (Nashua, NH). Resting

and daily metabolic rates (with concurrent activity levels) will also be measured every 2 weeks, and a suite of morphological measurements (girths, length, blubber thickness) will be taken twice weekly.

All blood sampling will performed under the supervision of the chief veterinarian at the Vancouver Aquarium Marine Science Centre. Blood samples are obtained via the caudal gluteal vein. Initially, animals are physically restrained for blood procedures. However, as soon as animals can be trained, gas anesthesia (isoflurane) will be used.

Blood samples are analyzed for 34 common parameters of blood biochemistry and hematology. For consistency, analysis is carried out by a qualified commercial facility, Central Veterinary Laboratories (Langley, BC, Canada). The consistency and scope of changes in blood parameters will be evaluated to determine which variables might prove to be effective bio-indicators of nutritional stress. Comparisons of variation in measures from healthy animals will also enable estimates of sample sizes required to identify nutritional stress among wild populations.

Concurrent with the imposed changes in nutrition we will be monitoring the reproductive hormones of the fur seals as they mature. In many mammals, periods of under-nutrition will disrupt the reproductive cycle (usually through anestrous). The role of short-term periods of under-nutrition on seasonal breeders is unclear, but is likely highly sensitive to the time of year that it occurs. Northern fur seals generally have their first pup at 5-6 years of age, although some can give birth much younger. The development of this reproductive potential, and the impact of periods of under-nutrition are unknown. While there are no plans to breed the female northern fur seals in captivity, we will be able to monitor key the development and cycling of reproductive hormones in non-pregnant females as they mature, and examine the effects of unpredicted periods of food restriction. This type of data will help link changes in individual physiology to changes in population-driving life history parameters.

These experiments will yield data on changes in blood biochemistry and body composition during short periods of under-nutrition. They will help to determine the physiological consequences of under-nutrition to individual fur seals and the efficacy of using body condition and blood hematology as accurate biomarkers of nutritional stress.

f. Determine the maximum food intake levels of young northern fur seals and their ability to alter intake to compensate for changes in food quality and availability

Bioenergetic models have proven to be a critical tool to understanding the relationship between pinniped behavior, food intake, and energetic requirements (Ryg and Øritsland, 1990; Winship et al., 2002). While a central parameter of many of these models is levels of food intake, little consideration is given to the actual ability to alter its daily food intake to compensate for changes in food quality or availability, nor the physiological constraints that might limit this ability. Chief among these constraints is an animals' maximum digestive capacity (Weiner, 1992). Experiments with captive Steller sea lions (Rosen and Trites, 2004) have established estimates of maximum daily food intake and the potential for compensatory food intake in response to changes in food availability and quality. These estimates have been critical parameters in creating realistic individual-based foraging models for this species. These models are used to understand the effect that changes in biotic and abiotic parameters have on sea lion behavior and, ultimately, population dynamics.

Given the increasing interest in fur seal bioenergetics, it is critical to determine maximum daily food intake and the potential for compensatory food intake in response to changes in food availability and quality for this species. Digestive capacity is an innate anatomical feature that can be modified by several factors, including age, season, and short-term consumption history. We will measure the voluntary food intake of young fur seals given *ad libitum* access to high energy or low energy prey on either a daily or alternate day schedule (see Rosen and Trites, 2004 for details). Maximum digestive capacity will become apparent as required food intake increases with decreases in feeding frequency and prey quality. Each phase (incorporating the 4 prey by availability combinations) of the experiment will last 24 days, and will be repeated 4 times during the year to test for seasonal and developmental changes.

The animals will be given food in a water-filled feeding trough. The trough will be monitored so that it is constantly full of fish. A complete change of fish and water will also occur every 90 min to minimize any spoilage. The troughs will be situated in a back area, free from any influence of trainers or researchers. The only contact during the 8 hr that the food is available will be researchers refilling/emptying the troughs with a net (to minimize performance-induced changes in food intake). Each day the amount of food removed from the troughs (corrected for water absorption) are subtracted from the total input to calculate daily food intake levels.

There are 2 experimental variables (prey quality and feeding frequency), with 2 levels each, that combine for 4 treatment conditions. The test prey will be high-energy herring and low-energy capelin, chosen for the differences in proximate composition but similarity in size. These will be offered either every day for 5 days in a row, or for 9 days but offered only every other day. Discarding the first day of these phases yields 4 feeding days for each level of prey availability. A cross-over design ensures that all animals do not receive the same treatments in the same order.

The results of these studies will illustrate how readily young fur seals are able to alter food intake levels to compensate for differences in food quality or availability, and provide realistic estimates of maximum food intake levels. This data will provide insights into the true impact of alterations in prey abundance or type on fur seal energy budgets, and establish realistic parameters for fur seal bioenergetic and foraging models.

g. Determine the species-specific calibration coefficients (enrichment values) needed to determine diet from fatty acid signature analysis and

h. Quantify digestion and recovery correction factors required to accurately describe diet from hard fecal remains (scat analysis) and

i. Determine the effectiveness of using stable isotope and fatty acid signature analyses to determine diet in wild fur seals

Understanding the relationship between diet and population tends requires a means to accurately describe the prey of northern fur seals in the wild. The traditional practice of direct analysis of stomach contents has been generally replaced by analysis of fecal hard parts, which has its own inherent limitations and biases. A number of alternate techniques have been recently developed to accomplish this task – fatty acid and stable isotope signature analyses, and genetic tissue identification. However, these techniques have only recently been subject to extensive testing and quantification of inherent biases. It has become obvious that most of these techniques require species-specific calibration data to accurately reconstruct food intake. For example, failure to account for differential recovery and digestion rates of fish otoliths and squid beaks will lead to large biases in diet reconstruction for species consuming both types of prey (Tollit et al., 2003). Also, species-specific calibration coefficients (CC's) for fatty acid signature analysis have been shown to be necessary to accurately describe diets using the QFASA technique (Iverson et al., 2004; Budge et al., 2006).

The experimental protocols for these analyses are well established. Various dietary manipulations coupled with frequent scat collection and identification are required to determine the effects of prey type, food intake levels, and activity on recovery and digestion rates (which lead to correction coefficients). The same protocols are used for supplying material for determining the accuracy of using genetic analysis of soft prey remains in scat for diet reconstruction (Deagle et al., 2005).

Diet reconstruction through analysis of predator fatty acid signatures requires knowledge of the preferential absorption, biosynthesis, accumulation and metabolism of key fatty acids. This information permits the correction of fatty acid levels found in the predator to more accurately reflect prey input ratios. Previous experiments have shown that these calibration coefficients are highly species-specific. Therefore, studies to advance the potential for fatty acid signature analysis require more basic dietary manipulations in order to calculate species-specific CC's. A series of experiments will utilize blubber samples from animals consuming different diets to calculate calibration coefficients. Initial studies will require the animals to be placed on longer term (~120 d) uniform diets of single, important prey species. Given the prevalence of squid in northern fur seal diets, two sets of experiments will be conducted with squid and pollock. Blubber samples will be obtained through standard biopsy techniques under supervised anesthesia. Serial blubber samples will yield the required CC's and also give estimates of turnover times. Once CC's are established, additional experiments will utilize species-appropriate mixed diets to evaluate the ability of the QFASA models derived from pure diets to predict more realistic feeding regimes.

ii. Marine Mammal Parts or Specimen Samples:
Approximately 10 mL of blood will be drawn on three separate occasions to determine initial health. Fecal loops will also be taken to assess parasite load. Samples will be analyzed on site for clinical hematology, blood biochemistry, and parasite loads. No samples will be transported off St. Paul Island.

3. Additional Information for Removing Animals from the Wild into Captivity

a. Requirement for Removal:

This permit proposes removing 6 pups permanently from the wild to form the core of a captive northern fur seal research colony. The aim of this research program is to directly address questions that can lead to the recovery of the species. This research is largely guided by the recommendations of the National Marine Fisheries Service Final Conservation Plan for Northern Fur Seals (NMFS, 1993). This includes

work on the possible role of nutritional stress, and the impact of biotic (fish type and distribution, disease) and abiotic changes (climate change) on northern fur seal populations.

A great deal of research has been completed on the nutrition and physiology of otariids (primarily on Steller sea lions). A number of otariids are held in public Aquariums and research facilities. Unfortunately, previous research has shown that the physiological responses of pinnipeds are highly species-specific, and little work has been conducted on basic fur seal physiology, nutrition, and bioenergetics. Therefore, northern fur seals must be used in the proposed experiments for the results to aid in the recovery of this species. However, the knowledge we have previously gained in studying otariids in captivity will be used to refine and optimize our experimental designs to obtain results in a timely and efficient manner.

Northern fur seals are currently kept at 3 institutions in North America: New York Aquarium, Mystic Aquarium, and the Seattle Aquarium. These institutions consider these animals as part of their permanent display collection, and are unlikely to give them to another institution for research. Even if feasible, the age and reproductive status of the animals would not be appropriate for the planned studies, given our current scientific focus of the effects of nutritional stress on younger animals. Most of the animals are older individuals, and some of the males have been neutered.

Young pinnipeds frequently come into rehabilitation centers along the western seaboard, and could potentially provide young northern fur seals that could be used for the proposed experiments. However, young stranded northern fur seals are rare and transfers to permanent research facilities are often against the mandate of rehab centers.

We have chosen to remove these animals from the population on the Pribilof Islands. Although the population at Bogoslov is growing, it is significantly smaller than the Pribilof population and is logistically more difficult to access. The greater logistical support on St. Paul will ensure that pups are captured with minimal disturbance and are provided optimum care. San Miguel (CA) is another potential source of fur seal pups. However, the genetic relationship between these animals and those from the declining population are unknown and might be a potential confounding variable in the interpretation of study results.

We have chosen to only take female pups to increase the scientific integrity of our studies. Any combination of sex ratios that might be considered would be insufficient to discern gender differences in study results. The survival of females and their young in the wild appear to be key to population trends of this species due to their polygamous breeding system. Data collection will also be facilitated by the smaller

size of females and the tendency for females to be less aggressive than males. Finally, investigations of female reproductive hormones and energetics as the animals mature are expected to provide important information needed to resolve scientific questions pertaining to the decline and recovery of northern fur seals in Alaska.

b. Transportation:

Animals that pass the initial health selection process will be held near the rookery in a specially built enclosure. A 12 x 10 foot area will be enclosed by a temporary fence, with the option of dividing the enclosure into two sections for either animal separations or to provide an enclosed work area. The enclosure will be manufactured from materials that are free from sharp edges, and high enough to prevent escape. A small, shallow pool will also be provided for thermal relief and play. During sessions when the animals are being evaluated for fish handling, this pool will be used as a food trough.

The 6 animals that make the final selection will be transported to the St. Paul airfield by truck, and then by chartered plane to Vancouver, B.C., accompanied by a veterinarian (Dr. Chris Harvey-Clark) and support staff. They will be transported in large (32" x 22" x 23") Vari-Kennel canine kennels (approved for airline transport, designed for maximum airflow and minimum transport myopathy) that are reinforced to prevent escape. The animals will not have been fed for at least 8 hr prior to transport (to minimize gut fill). The animals will be kept in close proximity to each other, as they are likely to have become socialized during their time in the beach enclosure. However, only one pup will be held in each kennel to avoid inadvertent injuries and overheating. Several additional precautions will be taken to minimize the risk of thermal stress during transport; water misters, battery fans, ice, and shading prior to loading on the airplanes. Transportation will also be conducted by at least 3 additional veterinary and husbandry staff experienced in pinniped health and handling, supervised by Dr. Chris Harvey-Clark.

Upon arrival in Vancouver the animals will be transported by truck to the Vancouver Aquarium Rehabilitation Center for a 30-day quarantine period. They will subsequently be transferred to the Marine Mammal Species-At-Risk Laboratory, which is in the off-display area of the Vancouver Aquarium. This area has 4 pools (minimum 10 foot depth) with ample haul out space, as well as numerous dry holding areas.

c. to e. Not applicable.

f. Veterinary approval:

During capture, initial holding, and transport, veterinarian care will be provided by Dr. Chris Harvey-Clark (Director, UBC Animal Care).

During holding at the research facility the animals will be under the care of Dr. Harvey-Clark and Dr. Martin Haulena (Veterinarian, Vancouver Aquarium Marine Science Centre). A statement from Dr. Harvey Clarke is attached.

g. Care and maintenance of the animals:

Holding facilities:

The research area at the Vancouver Aquarium consists of five pools with the following dimensions. Haulout space is also listed. Additional runs and open space are also available for working with animals. All pools utilize ambient, filtered sea water on a flow-through system. Water quality is continually monitored by facilities staff for water chemistry, and fecal levels are measured weekly. The Vancouver Aquarium conforms to standards and is inspected by the Canadian Council for Animal Care. The Vancouver Aquarium is accredited by American Zoo & Aquarium Association, Alliance of Marine Mammal Parks & Aquariums, and Canadian Association of Zoos and Aquariums.

- Tank 1: 8.5' x 16.0'; depth 6.5'; haulout space: 65 sq ft.
- Tank 2: 30.2' x 16.0'; depth 6.5'; haulout space: 168 sq ft.
- Tank 4: 13.0' x 16.0'; depth 6.5'; haulout space 88 sq ft.
- Tank 5: 6.0 ft diameter; depth 7.0'; haulout 92 sq ft.
- Research pool: 50' x 50'; depth 10'; haulout 200 sq ft.

Personnel:

Trainers for the Otariid research program are recruited from the senior members of the VA Marine Mammal Dept, and paid under contract. The Aquarium employs a full-time veterinarian and qualified animal health technicians. The Aquarium's supervising veterinarian is ultimately responsible for the health of the animals, with continual feedback from training and research staff. All research conducted with the animals must be approved separately by Animal Care committees at both the Vancouver Aquarium and the University of British Columbia. Dr. Andrew Trites is the Director of the UBC Marine Mammal Research Unit and Dr. David Rosen directs the captive Otariid research program; both have extensive pinniped experience.

Food prep and storage:

All food given to the captive Otariids is designated 'human quality'. All prey items are analyzed for proximate composition and oxidative state. Vitamin supplementation (Mazuri Vita-Zu Mammal Tab) is employed prophylactically for all diets. Herring is used as the basic diet species for captive pinnipeds. Herring is caught yearly and flash frozen. Additional prey items are purchased through commercial vendors. All food is stored offsite at a commercial freezer facility, and weekly

shipments are made to the Aquarium where they are stored in a designated freezer in the Marine Mammal Dept. food prep area. Daily food is refrigerated overnight, and then thawed in prep sinks, where they are sorted for quality. All food intake is carefully monitored. All areas and equipment used for food prep is suitably disinfected between uses.

h. Breeding program. Not applicable.

i. and j. Disposition of Animals

We anticipate that the proposed research will take at least 4 years to complete, and that the animals will become a long-term scientific resource. We plan to maintain the captive group for an extended period. We do not plan to transfer the animals to other facilities, nor do we believe that it would be suitable or feasible to release the animals back into the wild.

4. Lethal Take:

No lethal take is proposed. Mortality during capture and restraint is very unlikely. However, if such an unforeseen event should occur, the maximum mortality would not exceed 1 pup.

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

The Canadian Government through the Department of Fisheries and Oceans (DFO) has jurisdiction over marine mammals in Canada. 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.

D. Research Effects and Mitigation Measures:

1. Effects

a. Effects of proposed activities:

The physiological effects of the proposed activities on individual seals will mostly be related to the stress associated with the initial capture. For animals restrained for the first two levels of investigation (gender and gross health exams) this will be relatively short term (< 20 and <60 minutes, respectively) before they are released back onto the rookery. Captures will be carried out with experienced personnel, which will help to minimize handling and restraint time. Every possible precaution will be taken during these procedures to ensure that no harm comes to the animal. No drugs or other substances will be administered to the animals, unless required to save its life while in our care (see following details on appropriate veterinary response). We anticipate that once the pups are either placed into the temporary

enclosure for further holding or returned to the rookery their physiology and behavior should return to normal.

For animals that are temporarily held in the beach enclosure for 5-7 days, there may be additional stress as the pups are handled for further health assessments and are fed frozen fish. However, this stress should also be minimal, as restraint time for biological sampling will be minimized, and this is the time in their life history when they are learning to forage on their own (i.e., any imposed period without food will mirror fasting periods in undisturbed animals).

Transportation of the animals from the Pribilof Islands to Vancouver is likely to induce some level of stress. However, the animals will be kept together in visual and auditory contact, and will be closely supervised by a veterinarian with experience in the transport of pinnipeds. Several measures (detailed previously) will be employed that transport myopathy does not occur.

We propose on permanently removing 6 female pups from the population. Given the low survival rates of animals at this age (<40%; Lander, 1981), and the annual pup counts on St. Paul Island (2004=122,825; NMFS, 2004a) we do not feel that these takes will have a perceptible impact on the population.

b. Effects on non-target animals/species:

Some disturbance to nearby fur seals will occur during the captures, but we envision no disturbances to other vertebrate species. Incidental harassment of individual seals close to those being targeted for capture will be of short duration (i.e. < 30 min), and occur only a maximum of two times in each of two days. The slow crawling approach technique (previously described) minimizes the number of fur seals that are harassed in this way. Those animals directly in the approach path are able to move away in a slow relaxed manner and thus it is possible to avoid panic “running”. The same technique also makes it possible to approach the subject closely prior to capture, thus minimizing the number of nearby seals disturbed. In addition, once captured, the target animal is moved away from the initial area, allowing any incidentally harassed seals to quickly resume normal activities. Finally, restraint of the captured seals will occur out of line-of-sight of the group so that additional disturbance does not occur.

2. Measures to Minimize Effects:

Indirect disturbance:

Techniques employed for capture, evaluation and transportation will be those shown to be safe, effective and humane. The aim when working with groups of fur seals is that they do not climb on top of each other, overheat or suffocate.

Incidental disturbance will be kept to a minimum by taking precautions to avoid harassing seals wherever possible. These include maintaining a low vertical profile, moving well around groups of seals not involved in the research activities, identifying potential target animals beforehand from afar, coordinating our captures with concurrent research disturbances, and working as efficiently as possible to minimize the time spent on the rookery (per capture episode and in total). The behavioral response of a fur seal from incidental disturbance may range from turning and orienting toward the research activity to movement away from the research activity. If they move away from the rookery area, fur seals are expected to begin returning to the rookery or haul out area within 15 to 30 minutes after the disturbance. Time spent away from the rookery by adult northern fur seal females with pups after a disturbance ranges from 0.0 to 2.5 hours and abandonment of the rookery has never been observed (Gentry et al., 1977).

Captures will also be distributed across different peripheral regions of the rookery to ensure that no single area is overly disturbed. This can be accomplished on St. Paul Island because of the many potential areas to choose from.

Direct disturbance:

Prudent steps will be taken to minimize mortality as a result of our activities on the rookeries. A qualified veterinarian will be on St. Paul Island and available for assistance during all of our research activities. Only experienced researchers who are knowledgeable in detecting signs of stress in fur seals will be assigned the responsibility of actively working with the animals. The proposed methods of capture and restraint have been optimized to impose the least potential harm to the fur seals being captured as well as to minimize disturbance to all surrounding animals.

Captures will be carried out using only previously experienced personnel. As described previously, the capture method involves a slow crawl towards the target fur seal, designed to minimize secondary disturbance to the surrounding animals. This technique also has the advantage of providing sufficient time to confirm the identity and status of subjects at close range, and to ensure that the mother is away from the area. Only healthy (by visual inspection) pups that have been previously identified as being old enough but either newly weaned or near weaning will be chosen. Weaning occurs in the fall and is abrupt with either the mother abandoning the pup — or the pup abandoning its mother.

Once captured, the pup will be carried inland away from the surrounding fur seals (ca. 10-20 m) so that the population can quickly return to a normal state. Although capture myopathies have not been documented

to occur in pinnipeds, captured animals will be carefully monitored for signs of stress. If a captured animal shows signs of acute or protracted alarm reaction (e.g., overexertion, constant muscle tensions, abnormal respiration or heart rate) that may lead to serious injury, capture myopathy, or other disease conditions, or death, activities will be immediately suspended. Several actions will be taken immediately under the direction of the supervising veterinarian from the University of British Columbia. This includes loosening of restraints, and providing physical stimuli (flipper and ear pinches, muzzle slaps, body shakes). In the very unlikely event there is still no response, the seal can be intubated and manually-ventilated, receive CPR, and be administered appropriate cardiac-stimulating drugs (atropine, 0.02 mg/kg and epinephrine 0.01 mL/kg, delivered IV or inter-tracheally) by the attending veterinarian. If upon recovery (>60 min) the seal remains sluggish in behavior, it will be released back to the rookery, and observed for at least 3 days for further similar intervention.

The multi-stage process of selecting individuals for long-term holding will minimize the potential stress on any 'inappropriate' individual. Gender determination (stage 1 decision) can be done almost instantaneously once the animal is safely restrained. Gross physical exams (stage 2 decision) have been streamlined and clearly defined so that questionable individuals can be returned to the rookery as soon as possible. No additional measures will be taken from 'inappropriate' animals to further minimize their capture time. To the maximum extend practical without causing further disturbance of the rookery/haulout, animals are monitored post-handling for signs of acute stress or injury.

Restraint of the 8 fur seals destined to undergo more intensive examination will be minimized by dividing required tasks among team members, including one husbandry/vet staff assigned solely to animal monitoring. This will be aided by a (commonly used) restraint board. The first set of examinations and sampling will be carried out during the initial capture to minimize the number of captures, which is likely more potentially stressful than the total restraint time.

Field-sterile techniques are always utilized when collecting biological samples. Sterile, disposable needles are always used for blood sampling and injections of emergency drugs. Non-disposable items (endotracheal tubes, etc) are thoroughly disinfected with a bacteriocidal/virucidal agent in accordance with the product directions between animals.

When blood sampling, needle insertions do not exceed 4 attempts (needle insertions) per animal, and not more than 5 mL of blood per kg body mass is drawn per capture event. An additional measure to mitigate stress is introducing human contact to the fur seal pups held in the temporary enclosure as slowly as feasible.

3. Monitoring Effects of Activities

Individual animals that are held in the enclosure will be continuously monitored. Animals captured but returned to the rookery will also be monitored for 1 hour to ensure that no intervention is required to deal with stress-related effects directly attributable to the capture. Captured animals that are exhibiting symptoms of shock after release will be recaptured and receive appropriate veterinary treatment. The rookeries will also be monitored to estimate the overall effects of the capture disturbance.

4. Alternatives

Any stress, pain and suffering caused to pups will be a function of capture and restraint and final transportation. Unfortunately, there is no viable alternative to capture and restraint for determining the best pups, and getting them to an appropriate research facility. Minimizing the handling time of all captured seals is the best way to minimize any negative impacts caused by these activities. The University of British Columbia Animal Care Committee and scientists at the Alaska Fisheries Center have been consulted in applying for this permit to ensure that the proposed procedures are humane and appropriate to the research objectives. A copy of the UBC Animal Care permit (A06-1432) is attached.

E. Resources Needed to Accomplish Objectives

Copy of relevant Research proposal (approved) is attached.

F. Publication of Results:

The principal investigators involved in this study have a strong record of publishing their findings in leading research journals. We anticipate the core findings will appear in graduate theses and a series of primary publications. We have a proven track record of publishing innovative findings from studies of captive Steller sea lions. Possible journals are Marine Mammal Science, the Canadian Journal of Zoology, and Physiological and Biochemical Zoology. Preliminary results will be presented within the first year of research at conferences and symposiums. Additional information about the study and findings will also appear on our web site (www.marinemammal.org).

V. National Environmental Policy Act (NEPA) Considerations:

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

   No. All techniques (including the research proposed in Vancouver) have been previously carried out on other species of marine mammals by this research group.

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

   The planned activity includes blood and fecal sampling. Although the risk of zoonotic transmission is very low, standard clinical procedures will be employed to minimize these risks (disinfecting of injection and blood sampling sites, latex gloves for handling/processing all biological samples). No biological samples will, be transported off of the island.

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

   The U.S. Fish and Wildlife Service administers bird cliffs on St. Paul Island, and Otter and Walrus Islands as part of the Alaska Maritime National Wildlife Refuge. National Marine Fisheries Service administers the fur seal rookeries. The captures will not take place within the Alaska Maritime Wildlife Refuge, and will not impact the physical environment.

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

   The capture of female pups will not affect the subsistence harvest of male fur seals, and will not affect entities listed in or eligible for listing in the National Register of Historic Places, or cause destruction of scientific, cultural or historic resources.

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

   Pups undergoing preliminary examination will be transported about 10-20 m away from the rookery, and then returned if deemed inappropriate. Eight pups will be held in the temporary enclosure situated ~40 m from the beach. Two of these animals will be returned to the rookery after 5-7 days.

Equipment used in the captures and within the enclosure are either disposable or are thoroughly disinfected with an appropriate bacteriocidal / virucidal agent. We do not feel that transportation of animals between these sites presents a danger of introducing novel biological agents to the rookery.

VI. PREVIOUS AND OTHER PERMITS

A. Previous Permits:
Permit 715-1792 was assigned for Steller sea lion research (wild).

B. Other Permits: All research carried out under this permit application must also be covered by permits issued by the UBC Animal Care Committee. No other permits are required.

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VI. Certification and Signature:

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

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

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

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

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

Signature of Applicant:	Date of Signature:
	January 15, 2007
_____	_____

Andrew W. Trites, Ph.D.

Research Director
North Pacific Universities Marine Mammal Research Consortium

ATTACHMENT: Proposal to Funding Agency (approved)

Physiological Studies of Captive Northern Fur Seals

Principal Investigators:

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Project Period: July 1, 2006 – June 30, 2007

SUMMARY
Studies of numerous marine mammal species in the North Pacific indicate that observed population changes in the last 3 decades are symptomatic of ecosystem-wide changes. This project will establish a captive northern fur seal research program to expand the scope of scientific investigations into the effects of changes in the North Pacific ecosystem on marine mammal populations. The research program will conduct studies on trained animals to elucidate the reasons for the population decline and to formulate science-based plans for species recovery. Specific experiments will investigate aspects of fur seal physiology, nutrition, and bioenergetics.

INTRODUCTION
Several species of marine mammals in the North Pacific have experienced drastic population declines in the last decades, including harbor seals, Steller sea lions, sea otters, and northern fur seals. The northern fur seal population on the Pribilof Islands (St. Paul and St. George Island), in the central Bering Sea, is the largest U.S. population with an estimated 973,000 animals (York et al., 2000), which is approximately 70% of the world's population. The Pribilof Island rookeries are the principal breeding areas for this species (NMFS, 1993; Gentry, 1998; York et al., 2000). Population declines starting in the mid-1970s, and in 1988 it was determined that northern fur seals were below their optimum sustainable population level and were listed as "depleted" under the Marine Mammal Protection Act. Although this population has been apparently stable for some time, it has begun to decline again (Angliss et al., 2001). During 1998 to 2002 pup production has declined by 5.25% per year (SE = 0.19%, P = .02; York, 2002). In contrast, pup production on Bogoslof Island is increasing at a rate of 59%/year.

The causes for the continued population decline and lack of recovery are unknown. There are several environmental and anthropogenic factors that may affect population trends of northern fur seals. These include, but are not limited to, large-scale oceanographic climate change, fishing vessel disturbance, shift in prey species distribution and abundance, competition with fisheries, interactions with commercial fisheries, and subsistence harvesting (Angliss et al., 2001). Anthropogenic impacts may affect the population both directly (e.g. physical damage, disturbance) and indirectly (e.g. association with fishing activity); however there is little direct evidence supporting this (Richardson et al., 1995; Gentry, et al., 1998; Gisiner, 1998).

The apparent synchrony between the unexplained decline of northern fur seals and other North Pacific species, coupled with apparent changes in North Pacific fish populations and oceanography, has led to the hypothesis that nutritional stress may be a contributing factor to these population declines. This nutritional or energetic imbalance may be induced by changes in the distribution or abundance of prey, by either decreasing the energetic value of dominant prey or changing the costs of foraging or other activities. Diet studies indicate that fur seals in the Pribilofs consume primarily pollock and squid (Sinclair et al., 1994). Given the generally low caloric density of these prey, it would not take a great change in either prey intake or energetic expenditures to place the animals in energetic imbalance. This likelihood is increased by the potential shifts in fishing efforts away from Steller sea lion habitat towards fur seal foraging grounds. Given the highly specific home ranges of these animals, localized depletion may become a significant factor (Loughlin et al., 1987).

We propose to capitalize on the expertise gained in our current Steller sea lion captive research program by establishing a parallel captive research program with northern fur seals. The program will perform vital, controlled experiments with trained fur seals that would be impossible to perform with wild animals. This research will directly contribute to our understanding of northern fur seals, and diversify the scientific interests of the Consortium in response to changing scientific and financial interests.

OBJECTIVES

- Establish a captive northern fur seal research program to conduct studies of physiology, bioenergetics, and nutrition, and to develop techniques that can be used to study wild fur seals.
- Obtain baseline measures of growth, resting metabolism, and daily energy expenditures in young northern fur seals to enhance predictive bioenergetic models.
- Determine the fasting capabilities of young fur seals, and its interaction with thermal demands.
- Establish blood biochemistry and hematology parameters that can be used as bio-indicators of nutritional stress in northern fur seals.

- Determine the pattern of tissue catabolism during periods of under-nutrition.
- Estimate the maximum food intake levels of young northern fur seals.
- Determine the species-specific calibration coefficients (enrichment values) needed to determine diet from fatty acid signature analysis.
- Quantify digestion and recovery correction factors required to accurately describe diet from hard fecal remains (scat analysis).
- Determine the effectiveness of using stable isotope and fatty acid signature analyses to determine weaning period in wild fur seals.

STATEMENT OF WORK

Rational for these research projects:

To evaluate the potential impact of changes in the environment on young northern fur seals, it is important to understand key aspects of their bioenergetics, particularly in terms of energy partitioning under conditions when energy intake is insufficient to meet normal energy requirements. Pinnipeds have a variety of means to adjust to the type unpredictable changes in food intake proposed by the Nutritional Stress hypothesis, such as changes in body composition, metabolism, activity, and thermoregulatory capacity. However, the actual response of an individual depends on a number of factors including species, age class, reproductive status, time of year, and current health status. Therefore, controlled experiments are required to determine the likely physiological effects of restricted food intake on northern fur seals, taking as many of these variables into account as feasible.

For example, traditional fasting physiology theory suggests that under conditions of restricted energy intake, pinnipeds should primarily catabolise their subcutaneous blubber layer to provide additional energy. However, this layer must also be conserved as a thermoregulatory defense. While core tissues can provide energy, the sacrifice of key tissues will lead to long-term physiological and anatomical problems, and eventually death. Previous research on young Steller sea lions suggests that animals lose a surprisingly high proportion (40-50%) of core tissues during periods of energy restriction and body mass loss (Rosen and Trites, 2005). It is important to understand the effects of energy restriction on body composition, as it has both drastic immediate (thermoregulatory capacity, survival) and long-term effects (e.g., growth, size at maturity, reproductive success).

The high rate of core tissue loss exhibited by Steller sea lions during periods of mass loss may be a result of their relatively thin blubber layer compared to phocid seals. Given the climate of the North Pacific and Bering Sea, pinnipeds from this region would be likely to primarily conserve blubber for its thermoregulatory benefit. This would be particularly true for young northern fur seals with their greater surface area to body mass ratios and relatively sparse hypodermal blubber layer. In these animals, even slight changes in their lipid reserves may have major consequences to their overall energy budget through

increased thermoregulatory costs. Animals can often limit their mass loss by decreasing their metabolism, a physiological response known as metabolic depression. However, metabolic depression can diminish thermoregulatory capacity, as the individual is not producing as much heat to maintain core body temperatures. Although aspects of the thermoregulatory capacity of northern fur seals has been previously studied (Ohata and Miller, 1977; Donohue et al., 2000), their ability to respond to simultaneous energetic challenges (thermal and nutritional stress) is unknown. The way an animal balances these factors affects its activity, foraging decisions, and ultimately survival. Therefore, it is important to investigate the interaction between body composition changes and thermoregulatory capabilities during periods of restricted energy intake.

Alternately, an animal subject to decreased prey quality or availability may increase food intake when sufficient prey is available. However, the digestive system represents a physiological limit to such compensation (Weiner, 1992). This is particularly true for young animals that generally have higher relative energy requirements, smaller digestive systems, and decreased foraging (diving) capabilities. Quantifying the digestive capacity of captive Steller sea lions has helped to delineate ecological scenarios where increasing food intake is not a physiologically realistic strategy (Rosen and Trites, 2004). Similar measures of northern fur seals are critical to understanding the physiological attributes that may lead to nutritional stress.

Of course, a primary requirement of these studies is an ability to accurately describe the diet of northern fur seals in the wild. A number of techniques have been developed to accomplish this task in pinnipeds – fatty acid and stable isotope signature analyses, fecal hard part remains, and genetic tissue identification. Only recently have these techniques been subject to extensive testing and quantification of inherent biases. It has become obvious that most of these techniques require species-specific calibration data.

From a practical viewpoint, it is also critical to determine whether nutritional stress is apparent in different segments of the wild fur seal population at different times of the year. Analysis of blood samples have proven a valuable tool for wildlife managers for a number of mammals. Unfortunately, changes in blood profiles are species-specific and depend partly on the duration and severity of nutritional stress. We propose to develop simple physiological indicators of nutritional stress. This includes tracking changes in blood biochemistry and hematology, and body composition indices during periods of restricted food intake. Accounting for natural variation in these parameters is also critical to calculating approximate sample sizes required to detect these differences between populations. These results can then be applied in the future to samples obtained from wild northern fur seals.

While much physiological research can be undertaken on animals in the wild, the ability to conduct long-term controlled manipulations are limited. Research

programs with dedicated populations of captive marine mammals have been instrumental in scientific breakthroughs that would be impossible with animals in the wild.

Methodology:

Establish a captive northern fur seal research program

We hope to establish the research colony in Fall 2006. Animals will be obtained from St. Paul Island in the Pribilofs, after consultation and with the co-operation of local interest groups and obtaining the necessary federal permits (an initial permit has already undergone preliminary review and comment).

Initial takes and holding/evaluation will be for two weeks in October 2006 on St. Paul Island (57° N, 170°W) in the Bering Sea, Alaska. St. Paul Island is a fur seal rookery. Unlike the technique used to establish captive Steller sea lions, the smaller size and younger age of northern fur seals at this stage makes it logistically easier to wait until the animals are nearly or newly weaned before capture. With the assistance of NMFS researchers, we propose to remove 8 female pups that are approaching weaning from the fringes of the rookery. Two possible rookeries are Vostochni (northeast rookery) and either English Bay or Reef (southwest rookeries).

Prior to captures, observations will determine which pups are old enough to potentially wean (based on size), but are still nursing, and perhaps even gender and health status. Location and naturally occurring marks and brands (both mothers and pups) will be used to identify individuals. Observations will also determine when females are away from their pups before capture attempts. Captures begin by slowly crawling towards the subject while making maximal use of the local topography (e.g. boulders) to allow close approach without being detected. Such an approach minimizes disturbance to any surrounding fur seals while getting within capture range of a potential subject, and at the same time provides the opportunity to observe and confirm the subjects. Pups will be captured with a reinforced hoop-net (ca. 0.8 m diameter) and carried a short distance away (e.g. 10-20 m; out of sight from other animals). In order to obtain the 8 initial subjects, we have allowed for a total of 16 pups to be temporarily disturbed for up to 30 minutes. This will provide time to ascertain gender, age, and health status.

The animals will be held within a specially-built enclosure near the rookery, where initial health and suitability testing will take place. Animals will initially be fed in water-filled feeding troughs (to minimize imprinting), using previously frozen herring and capelin. Over the next 5-7 days, the group will be decreased to 6 fur seals, based on health parameters (physical examinations and blood parameters analyzed on site and inclination to consume dead fish. The 2 “rejected” fur seals will be placed back in the rookery where they were obtained; they will still be young enough to resume their normal life history. The remaining 6 animals will be transported by air to Vancouver, Canada. Animals transported

to Vancouver will be housed in the Marine Mammal Species-At-Risk Research Laboratory at the Vancouver Aquarium Marine Science Centre after a 30-d quarantine period. During this period the pups will be introduced to hand feeding. These animals will undertake long-term research projects for at least 4 years. Although the possibility of reintroduction exists, these fur seal will be highly trained individuals that are expected to be permanently housed at the MMSAR Laboratory for critical research participation.

Obtain baseline measures of growth, and resting and daily metabolism

Previous bioenergetic models of pinnipeds have indicated several key variables of energy requirements that can be best parameterized through longitudinal studies of captive animals (e.g., Winship et al., 2002). These include growth rates and efficiency, resting metabolism, and daily metabolic rate. Body mass and food intake will be measured daily, and animals will be subject to a set of morphological measurements twice a week. These include total body length, body girth at 3 locations along the trunk, and dorsal and lateral blubber depth at these same locations. Blubber depth is determined with a Sonosite 180 machine. Body composition will also be determined every 2 months using deuterium dilution techniques (Reilly and Fedak, 1990), and conversions from Arnould et al. (1996). The proximate composition and total energy content of all prey items are also recorded. Resting metabolism is measured for 90 minutes every 6 weeks using indirect respirometry in the dry metabolic chamber, and daily metabolic rate is measured over 24 hr in the large metabolic tank every 6 weeks.

Fasting capabilities and the impact of concurrent thermal demands

Previous studies with young Steller sea lions have shown that these animals display rapid, substantial metabolic depression during short periods of fasting. This is presumably a defensive physiological adaptation to maternal foraging/attendance patterns. However, metabolic depression limits the capacity for thermoregulatory responses. In Steller sea lions, the responses to concurrent fasting and thermal challenges were dependent on age, initial body condition, and initial rate of mass loss (Rosen unpubl. data). This response is likely also affected by seasonal changes in energetic priorities. The nature of the response is a significant, complex interaction between climate, fur seal fasting capabilities, and individual foraging success.

We propose conducting experiments on the metabolic response of fur seals during a 48-hour fast, during winter 2006 and summer 2007. These experiments will provide data on the comparative ability of young northern fur seals to thermoregulate in cold water before and during a period of nutritional stress. The results will quantify the energetic costs of short periods of nutritional stress on young fur seals.

Resting metabolism will be measured at 0, 24, and 48 hr into the fasting period and 24 hr post-refeeding. Immediately following each measure of resting metabolism will be a measure of metabolism during a thermal challenge using a

specially designed metabolic chamber that can be partly filled with water at a set temperature. Metabolism is measured via gas respirometry (oxygen consumption rates) using Sable System (Henderson, NV) analysers and samplers. Body temperature will also be measured during and after the trials, and body condition will be determined at the start and end of each experiment using deuterium dilution techniques. Blood samples will also be obtained for clinical and experimental analyses.

Blood biochemistry and hematology as bio-indicators of nutritional stress and
Pattern of tissue catabolism during periods of under-nutrition
Studies with captive Steller sea lions have indicated that, contrary to the previously-established pinniped model, these animals utilize a large proportion of core tissues during periods of energy restriction and body mass loss (Rosen and Trites, 2005). While this might be a species-specific response, it is more likely a general Otariid strategy resulting from their relatively limited lipid reserves (compared to phocid seals). During these experiments, we also identified a subset of blood parameters that appear to be viable biomarkers of nutritional stress (Rosen et al., 2004).

We propose to conduct experiments to determine the effects of food restriction on body lipid stores and other aspects of tissue catabolism and blood profiles in northern fur seals. The food intake of the animals will be initially monitored over a 28-d control period. The animals will then be held on submaintenance diets, designed to lose 10-15% of their body mass over 42 days. This is followed by a 28-d recovery period where the animals receive the same quantity of food as they consumed during the control period. This phase of the experiment is designed to determine the scope and mechanisms of compensatory growth. During a final 28-d ‘ad lib’ phase, the animals will be allowed to consume as much food as their training protocol permits.

Blood samples will be obtained and body composition will be determined through deuterium dilution every 2 weeks during the entire experiment. Serum and dose samples for the deuterium dilution technique are analyzed by Metabolic Solutions (Nashua, NH). Resting and daily metabolic rates (with concurrent activity levels) will also be measured every 2 weeks, and a suite of morphological measurements (girths, length, blubber thickness) will be taken twice weekly.

All blood sampling will done under the supervision of the chief veterinarian at the Vancouver Aquarium Marine Science Centre. Blood samples are obtained via the caudal gluteal vein. Initially, animals are physically restrained for blood procedures. However, as soon as animals can be trained, gas anesthesia (isoflurane) will be used.

Blood samples are analyzed for 34 common parameters of blood biochemistry and hematology. For consistency, analysis is carried out by a qualified

commercial facility, Central Veterinary Laboratories (Langley, BC, Canada). The consistency and scope of changes in blood parameters will be evaluated to determine which variables might prove to be effective bio-indicators of nutritional stress. Comparisons of variation in measures from healthy animals will also enable estimates of sample sizes required to identify nutritional stress among wild populations.

These experiments will yield data on changes in blood biochemistry and body composition during short periods of under-nutrition. They will help to determine the physiological consequences of under-nutrition to individual fur seals and the efficacy of using body condition and blood hematology as accurate biomarkers of nutritional stress.

Determine the maximum food intake levels of young northern fur seals

Bioenergetic models have proven to be a critical tool to understanding the relationship between pinniped behavior, food intake, and energetic requirements. While a central parameter of many of these models is levels of food intake, little consideration is given to the physiological constraints that might limit this value. Chief among these constraints is an animals' maximum digestive capacity. Experiments with captive Steller sea lions (Rosen and Trites, 2004) have established estimates of maximum daily food intake and the potential for compensatory food intake in response to changes in food availability and quality. These estimates have been critical parameters in creating realistic individual-based foraging models for this species. These models are used to understand the effect that changes in biotic and abiotic parameters have on sea lion behavior and, ultimately, population dynamics.

Given the increasing interest in fur seal bioenergetics, it is critical to determine maximum daily food intake and the potential for compensatory food intake in response to changes in food availability and quality for this species. We will measure the maximum voluntary food intake of young fur seals given ad libitum access to high energy or low energy prey on either a daily or alternate day schedule. Each phase (incorporating the 4 prey by availability combinations) of the experiment will last 24 days, and will be repeated 4 times during the year to test for seasonal and developmental changes.

The animals will be given food in a water-filled feeding trough. The trough will be monitored so that it is constantly full of fish. A complete change of fish and water will also occur every 90 min to minimize any spoilage. The troughs will be situated in a back area, free from any influence of trainers or researchers. The only contact during the 8 hr that the food is available will be researchers refilling/emptying the troughs with a net. Each day the amount of food removed from the troughs (corrected for water absorption) are subtracted from the total input to calculate daily food intake levels.

There are 2 experimental variables (prey quality and feeding frequency), with 2 levels each, that combine for 4 treatment conditions. The test prey will be high-energy herring and low-energy capelin, chosen for the differences in proximate composition but similarity in size. These will be offered either every day for 5 days in a row, or for 9 days but offered only every other day. Discarding the first day of these phases yields 4 feeding days for each level of prey availability. A cross-over design ensures that all animals do not receive the same treatments in the same order.

The results of these studies will illustrate how readily young fur seals are able to alter food intake levels to compensate for differences in food quality or availability, and provide realistic estimates of maximum food intake levels. This data will provide insights into the true impact of alterations in prey abundance or type on fur seal energy budgets, and establish realistic parameters for fur seal bioenergetic models.

Determine the species-specific calibration coefficients (enrichment values) needed to determine diet from fatty acid signature analysis and
Quantify digestion and recovery correction factors required to accurately describe diet from hard fecal remains (scat analysis) and
Determine the effectiveness of using stable isotope and fatty acid signature analyses to determine weaning period in wild fur seals

Understanding the relationship between deit and population tends requires a means to accurately describe the prey of northern fur seals in the wild. The traditional practice of direct analysis of stomach contents has been generally replaced by analysis of fecal hard parts, which has its own inherent limitations and biases. A number of alternate techniques have been recently developed to accomplish this task – fatty acid and stable isotope signature analyses, and genetic tissue identification. However, these techniques have only recently been subject to extensive testing and quantification of inherent biases. It has become obvious that most of these techniques require species-specific calibration data to accurately reconstruct food intake. For example, failure to account for differential recovery and digestion rates of fish otoliths and squid beaks will lead to large biases in diet reconstruction for species consuming both types of prey (Tollit et al., 2003). Also, species-specific calibration coefficients (CC's) for fatty acid signature analysis have been shown to be necessary to accurately describe diets using the QFASA technique (Iverson et al., 2004).

The experimental protocols for these analyses are well established. Various dietary manipulations coupled with frequent scat collection and identification are required to determine the effects of prey type, food intake levels, and activity on recovery and digestion rates (which lead to correction coefficients). The same protocols are used for supplying material for determining the accuracy of using genetic analysis of soft prey remains in scat for diet reconstruction (Deagle et al., 2005).

Diet reconstruction through analysis of predator fatty acid signatures requires knowledge of the preferential absorption, biosynthesis, accumulation and metabolism of key fatty acids. This information permits the correction of fatty acid levels found in the predator to more accurately reflect prey input ratios. Previous experiments have shown that these calibration coefficients are highly species-specific. Therefore, studies to advance the potential for fatty acid signature analysis require more basic dietary manipulations in order to calculate species-specific CC's. A series of experiments will utilize blubber samples from animals consuming different diets to calculate calibration coefficients. Initial studies will require the animals to be placed on longer term (~120 d) uniform diets of single, important prey species. Given the prevalence of squid in northern fur seal diets, two sets of experiments will be conducted with squid and pollock. Blubber samples will be obtained through standard biopsy techniques under supervised anesthesia. Serial blubber samples will yield the required CC's and also give estimates of turnover times. Once CC's are established, additional experiments will utilize mixed diets to evaluate the ability of the QFASA models derived from pure diets to predict more realistic feeding regimes.

One special aspect of QFASA will be investigated right from the data of capture: identification of weaning through fatty acid signature shifts. The date of weaning has proven to be a critical life history parameters for evaluating the sources of population shifts. Traditionally, weaning has been determined by either intensive behavioral observations or intrusive stomach content analyses. In theory, the dietary changes associated with weaning should be accompanied by a shift in both stable isotope and fatty acid signatures. Serial blood samples and whiskers from the pups as they are introduced to fish will be used to determine whether these dietary shifts can be readily identified from these samples.

Milestones/Timelines:
The most critical aspect of this project is obtaining permits to capture and transport animals by September 2006 (or the project will be delayed until Fall 2007). This will require immediate action for both the formal permit process and to obtain the co-operation of local interest groups in the Pribilofs. We have already received feedback on the initial permit draft from members of the NMFS Office of Protected Resources and the Research Committee. Research will commence upon capture, and intensify once they have been established in captivity.

BUDGET & NARRATIVE
Funds have previously been approved for this program ($106,248) for July 2006 – June 2007. Since that time, scientific developments have allowed an expansion of the scope of questions that should be addressed with the captive fur seals. Additional funds ($25,000) are requested for research supplies to carry out an expanded research program that will ultimately yield a greater scientific return on the Consortium's investment. The expanded list of specific experiments are itemized in the proposal. To accomplish these goals, we are requesting funds for

additional blood analyses, deuterium oxide analyses, fatty acid sample analyses, and experimental food.

PERSONNEL

Some of the research will be conducted by Dr. Rosen and an M.Sc student co-supervised by Dr. Trites. The student will also assist in much of the other data collection.

Dr. David Rosen is a Research Associate with the University of British Columbia. His areas of specialization are bioenergetics and physiology of marine mammals. His responsibilities are to supervise and co-ordinate the studies with the captive Steller sea lions, and to conduct his own studies.

Dr. Andrew Trites is the Research Director for the North Pacific Universities Marine Mammal Research Consortium. He is responsible for overseeing the priorities of the scientific program and for integrating the captive, field, and modeling research programs.

LIKELIHOOD OF SUCCESS

We anticipate that the productivity of the Northern fur seal captive research program will at least equal that previously demonstrated by the captive Steller sea lion research. It should yield at least 2 major publications and one thesis per year. Please see the attached CVs of the Principal Investigators for a sample of publications resulting from previous research.

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