American Zoo and Aquarium Association

TITLE OF PROJECT: Investigation of *Streptococcus bovis* complex and associated valvular endocarditis; a major cause of mortality in northern sea otters (*Enhydra lutris kenyoni*).

Principal Investigator (PI) Name and Title: Carrie Goertz, MS, DVM, Associate Veterinarian
Institution/Organization: Alaska SeaLife Center
AZA Individual Membership Level and No.: Affiliate No.
Institutional Address: PO Box 1329, 301 Railway Avenue, Seward AK 99664
Phone, FAX, E-mail: [Redacted], Carrie_Goertz@Alaskasealife.org

Collaborator Name and Title: Verena Gill, Wildlife Biologist
Institution/Organization: U.S. Fish and Wildlife Service Marine Mammals Management
Institutional Address: 1011 East Tudor Road, MS 341, Anchorage, Alaska 99503-6199
Phone, FAX, E-mail: [Redacted], Verena_Gill@fws.gov

Collaborator Name and Title: Pam Tuomi, DVM, Senior Veterinarian
Institution/Organization: Alaska SeaLife Center
Institutional Address: PO Box 1329, 301 Railway Avenue, Seward AK 99664
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Collaborator Name and Title: Angela Doroff, Wildlife Biologist
Institution/Organization: U.S. Fish and Wildlife Service Marine Mammals Management
Institutional Address: 1011 East Tudor Road, MS 341, Anchorage, Alaska 99503-6199
Phone, FAX, E-mail: [Redacted], Angela_Doroff@fws.gov

Collaborator Name and Title: Barbara Byrne, DVM, PhD, Assistant Professor
Institution/Organization: U.C. Davis, School of Veterinary Medicine, Department of Pathology, Microbiology, and Immunology, Chief, Vet. Med. Teaching Hospital Microbiology Lab.
Institutional Address: One Shields Ave. Davis, CA 95616
Phone, FAX, E-mail: [Redacted], bbyrne@ucdavis.edu

Collaborator Name and Title: Kathy Burek, DVM, MS, Diplomate ACVP
Institution/Organization: Alaska Veterinary Pathology Services
Institutional Address: 23834 The Clearing Drive (or P.O. Box 773072), Eagle River, AK 99577
Phone, FAX, E-mail: [Redacted]

Total Project Budget $ 48, 637.00
Projected Duration of Project: 1 Year
Is the project underway? (Y/N) No

Total Requested from CEF $ 19, 797.00
Dates for CEF Support (mo/yr) Start: 4/07 End: 3/08
Starting Date: Spring 2007
PROJECT REVIEW CATEGORY

Please select the two most pertinent topic areas for the project by placing an “X” next to each:

- [X] Field Conservation (*In situ*)
- — Management/Captive Breeding (*Ex situ*)
- [X] Animal Health
- — Conservation Education
- — Research
- — Animal Welfare

ABSTRACT

Alaskan sea otter populations west of Cook Inlet have recently undergone catastrophic declines in numbers, exceeding 90% in some areas, and this population is now listed as threatened under the Endangered Species Act. The role of disease as a factor contributing to the decline or constraining the recovery of sea otters in southwest Alaska has only recently been investigated. A better understanding of disease and patterns of mortality are essential to the proper management and conservation of this species. Preliminary data has shown a high prevalence of valvular endocarditis and concurrent *Streptococcus bovis- equinus* complex infection in beach cast northern sea otters, particularly in Kachemak Bay, AK. This study proposes to survey both wild caught and captive animals to determine the prevalence of the *S. bovis- equinus* complex organism in apparently healthy animals by examining the flora obtained from nasal, pharyngeal, and rectal swabs or fresh feces from these otters. We also plan to collect samples for serology to look for underlying causes of this condition and where possible, to conduct cardiac echocardiography to look for valvular changes. Samples from a target of 35 live otters captured from the affected wild population in Kachemak Bay will be supplemented with samples obtained from captive animals during regularly scheduled husbandry or medical procedures. Understanding the relationship between *S. bovis- equinus* complex and valvular endocarditis will help wildlife managers better manage the population.
1) Explain the goal and rationale behind the proposed project.

Sea otters were exploited to near extinction during the 18th and 19th centuries in the Pacific basin fur trade. After cessation of the legal hunting in 1911, sea otter populations began to recover and are now found throughout most of their original range, including Alaska and California. Recently, populations of northern sea otters (*Enhydra lutris kenyoni*) have demonstrated a decline (between 56 – 94%) in the Aleutian Islands, the Alaska Peninsula and the Kodiak Archipelago (Doroff, et al 2003). The southwest Alaskan stock is now listed as threatened under the Endangered Species Act. In the southcentral and southeast Alaska population stocks, sea otter numbers continue to remain stable, with occasional mortality events. (Doroff et al. 2003) Other marine mammal species, such as the western population of Steller sea lions (*Eumetopias jubatus*), have also been declining dramatically in the areas of sea otter habitat in Alaska over the last 20 years, beginning initially in the Aleutian Islands and spreading to southcentral Alaska (Loughlin 1998). If sea otters follow this same pattern, baseline information collected now will be extremely valuable in understanding causes of the decline and potential for intervention to aid in population recovery.

There is little information regarding the causes of mortality in the Alaskan sea otter population. Work focusing on the presence and prevalence of infectious disease in northern sea otters is important in assessing population changes and ecosystem health. Sea otters are an important sentinel species living in the near shore marine environment and consuming 25% body weight per day of prey, many of which are filter feeders such as shellfish that concentrate waterborne pathogens (Reidman 1998, Tamburini 1999).

For the past three years, collaborative work has been conducted between biologists and pathologists in Alaska and California comparing cause of death and other findings in beach cast carcasses of southern and northern sea otters. Results of necropsy examinations performed to date show that nearly 50% of 130 fresh carcasses collected in Kachemak Bay and lower Cook Inlet had mild to severe valvular endocarditis. Where cultures have been possible, these animals were identified as having infection due to *S. bovis-equinus* complex. Small numbers of carcasses have been collected from other areas in Alaska and at least two of these have had valvular endocarditis. In comparison, out of hundreds of California otters that have been necropsied, this infection has only recently been found in two animals. The source for this bacterium is unknown in either population although it has been suggested that infection may gain entry through the gastrointestinal tract and may be associated with the type of prey consumed. Discussions with the California researchers have led to the joint decision to add screening for *S. bovis-equinus* complex in northern and southern otters in order to be able to make comparisons between the presence of this organism in the two populations. Additionally, we plan to work with microbiologists to perform molecular comparisons of the isolates and attempt to identify the source of this bacterium as well as monitor changes in prevalence over time. Not only is this finding important for sea otter health but it may have an impact on human health research as *S. bovis-equinus* is an emerging human cardiac pathogen.

The *Streptococcus bovis/equinus* complex consists of Lancefield Group D, non-enterococcal streptococci. These Gram positive cocci exist in the gastrointestinal tract of many mammals including man and ruminants and have been associated with a variety of diseases. Most prominently, members of the *S. bovis-equinus* complex have been demonstrated to cause endocarditis, bacteremia, and septicemia in man, ruminants, pigeons, and mink.

The taxonomy of the *S. bovis-equinus* complex has undergone dramatic changes recently
with the identification of multiple biotypes and species and at least 4 distinct genogroups (Schlegel 2000, Clarridge 2001). These recent changes have illustrated the diversity of species within the complex. To further confuse the reader, most papers describing isolates from infective endocarditis have only identified isolates as *S. bovis* or a member of the *S. bovis* complex. Thus, comparison of isolates and understanding their relatedness between hosts has been difficult.

The origin of infection in infective endocarditis is uncertain in any species although it is thought that most arise from the gastrointestinal tract from transient or colonizing *S. equines-bovis*. Interestingly, a recent study has demonstrated a regional increase in endocarditis due to the *S. bovis* complex in humans (Hoen 2005). This finding appears similar to the differences seen in the northern and southern sea otter populations. A study evaluating the relatedness of *S. bovis* isolates from mink with endocarditis demonstrated that many isolates were highly related suggesting that there may be a common source or spread of related strains within a population (Pedersen 2003).

Recently, a member of the *S. bovis* complex has been isolated from cases of infective endocarditis and septicemia of the northern and southern sea otter species. Based on biochemical and genetic analyses these isolates belong to the species *S. infantarius* ssp. *coli* (Jang 2005).

A few of the *S. infantarius* ssp. *coli* isolates from infective endocarditis or septicemia in northern and southern sea otters have been compared using pulsed-field gel electrophoresis (Byrne 2005). Interestingly, many but not all of these isolates from the northern sea otter population appear to be highly related suggesting either a common source of infection or clonal spread within the otter population. The isolates from northern and southern sea otters do appear to differ, a finding consistent with distinct strains affecting each population. Additionally, isolates collected from the gastrointestinal tract, lymph node(s) or feces from a single affected animal are identical to that isolated from the heart of this animal. This finding is consistent with spread of the isolate from the gastrointestinal tract to the heart. These studies have been carried out on a limited number of isolates and none from normal animals. Additional isolates are needed from both affected and unaffected animals in order to determine the relatedness of *S. infantarius* ssp. *coli* in otters.

This proposed study brings together a unique team of Alaskan and California veterinarians and scientists who are experienced in wildlife studies and disease investigations. Increasing our knowledge of this emerging and potentially zoonotic disease in wild and captive sea otters will provide the groundwork for future studies, as well as invaluable data on current population status and health. This information will be directly applicable to monitoring and conserving endangered southern and threatened northern sea otters in the future.

Our project goals are:

1) Establish the prevalence of *S. bovis- equinus* complex in otherwise healthy animals, in both live captured wild sea otters and those in captivity.

2) Perform health assessment to include complete blood cell count and serum chemistries.

3) Evaluate the usefulness of ultrasound to identify valvular endocarditis in live animals.

4) Obtain opportunistic samples for other research projects (e.g. genetic samples).
2) Explain the methodology for this project and why it is appropriate. (For instance, if this is a research project, then clearly state the hypothesis to be tested; if this is a conservation education or professional training project, describe your target audience and how many people will be reached, etc.)

*Our Hypothesis:* Bacteria of the *S. bovis*-*equinus* complex are commonly present in the mucous membranes and gastrointestinal tract of healthy sea otters and are not necessarily associated with clinical disease, namely valvular endocarditis.

Bacterial cultures will be conducted on samples collected from the nasopharynx and gastrointestinal tract of live northern sea otters captured in the wild and a subset of those currently held in captivity. Concurrent health assessments will be performed on each animal to attempt to detect any signs of valvular disease or other infectious processes.

*Live Capture Methodology:* Thirty individual sea otters will be captured either in tangle nets, hand-held dip nets or under-water diver-held traps. These devices are commonly used in sea otter field research. Otters will be transported from their capture location via the capture skiff to a support vessel for sampling. During transport animals will be placed in a large ventilated capture kennel and provided with adequate fresh air and cold water.

Captured sea otters will be anesthetized using a combination of fentanyl citrate and diazepam and reversed with naltrexone hydrochloride. This combination has been used successfully for the anesthetization of well over 500 sea otters in Alaska since 1992, with only one drug related mortality (<0.2% of anesthetized otters) recorded in that time (Monson et al. 2001). Sampling will be initiated as soon as anesthesia allows. In the event that more than one animal is captured, additional animals will be held in either floating net pens or floating boxes, in cold seawater. Holding time will be minimized by processing all animals in a timely manner. Naltrexone, at a dose equal to 2X the fentanyl dose, which is drawn up prior to initial anesthetization (i.e. available for immediate reversal if necessary), and is administered intravenously and/or intramuscularly following handling. Following administration of the antagonist the otter is allowed to recover inside a capture box (usually 2 or 3 minutes), and released into the water when alert and active. Holding/handling times for an individual average about 30 minutes.

To prevent hyperthermia, sea otters are generally kept cool by holding in water. As soon as the otter is anesthetized a rectal temperature is taken and recorded, and appropriate preventative measures taken as required (e.g. ice cubes wrapped in wet towels and placed on flippers if above 38°C, or towel dried and covered if below 35°C). In addition, heart rate, respiration rate and capillary refill time are monitored periodically throughout the procedure.

All animals captured will be marked with a with unique color/number coded polyethylene "Temple Tags" in the hind flippers to be able to visually identify individuals (e.g. longitudinal studies) and to prevent repeated sampling of the same individuals.

*Biological Sampling:* Morphometric data will include age class, total length, weight, sex, girth, canine width and baculum length (in males). Morphological characters will include head color, length and tooth wear. One blood sample of up to 30 cc per adult otter, or up to 5% of the blood volume per juvenile otter may be collected from each sea otter. Kenyon (1969) found that sea otters are about 8% blood by weight, thus a 30 cc sample represents less than 2% of the blood volume of a typical sea otter. One premolar (Bodkin et al. 1997) will be removed for age estimation. Bacteriological samples will include nasal, pharyngeal, and rectal swabs and fresh feces and will be sent to the U.C. Davis School of Veterinary Medicine Microbiology lab for culture and identification. Ultrasound echocardiography will be performed by a qualified
veterinarian to image the heart valves. This can be done using water or alcohol to wet a small area of skin near the sternum without clipping or otherwise damaging the haircoat of the otters. Additional samples in support of other research projects may include collection of oral lesions for histopathological analysis, serum for serological analysis and biomarkers and whole blood for toxicology.

Release: All animals will be released alive and in good health following described procedures. Attending veterinarians will hold responsibility for the administration of proper treatment of study animals. Sea otters captured under the proposed activities will be released within two hours of marking. Mother-pup pairs will be released together. Researchers in the field will halt all capture activities if it is determined their activities are having a significant negative impact on the animals. A significant impact on the animals is defined as the mortality of more than two animals directly as a result of capture activities.

Preparation, storage, handling and transport of immobilization drugs: Preparation, storage, handling and transport of immobilization drugs will follow the procedures identified in the Fish and Wildlife Service controlled substance policy and the United States 21 CFR controlled substances act. The project leader will assume responsibility for the safe handling of immobilization chemicals. Locked transport boxes will be used for transport.

Captive Animal Methodology: There are approximately 20 northern sea otters in captivity at five different institutions. Samples consisting of nasal, pharyngeal, and rectal swabs or fresh feces will be obtained from all available animals during routine handling events over the course of the year and sent to U.C. Davis for culture and analysis. The use of physical restraint and or anesthetic agents will be left to the discretion of the staff veterinarian at the captive facility.

Bacterial Culture Methodology: S. bovis complex organisms will be isolated from pharyngeal and rectal swabs utilizing phenylethanol agar (PEA) blood agar plates. Alpha-hemolytic colonies identified as Gram positive cocci will be screened for catalase (negative) and absence of growth in 6.5% NaCl. Biochemical testing will utilize the API20 and/or Rapid STREP identification systems. Further confirmation of the Streptococcus isolates as S. infantarius ssp. coli will be confirmed with sequencing of the 16S RNA gene (Schlegel 2000). The laboratory of one of the investigators routinely screens samples from marine mammals for this isolate (Jang 2005).

Genotyping of S. bovis complex isolates: Pulsed-Field Gel Electrophoresis (PFGE) will be used to identify similarity between S. infantarius ssp. coli isolates using the methods of Vela et al. (2003) will be utilized for preparation of DNA in agarose plugs. The resulting banding patterns will be evaluated with BioNumerics software (Applied Maths) and interpretation of strain differences will utilize the methods of Tenover (Tenover 1995). Separate funding is being sought for this portion of the project.

Expected results and interpretation: If the isolates from endocarditis and septicemia are different from those found in normal animals, this suggests that a distinct and possibly more virulent strain of S. infantarius ssp. coli is responsible for disease. If the isolates in normal otters and those with endocarditis are similar, then it is more likely that the high incidence of endocarditis is resulting from increased susceptibility of some otters to invasion by this Streptococcus. Investigation of additional methods of genotyping and potential virulence factors would be pursued in future studies.
3) What is the conservation/management significance of the specific outcomes? Explain how the project is compatible with the purposes of the CEF.

Northern sea otters (*Enhydra lutris kenyoni*) in southwest Alaska have recently undergone a catastrophic decline in numbers, exceeding 90% in some areas, and this population is now listed under the Endangered Species Act. The role of disease as a factor contributing to the decline or in constraining recovery in southwest Alaska is unknown. Preliminary data indicate that valvular endocarditis associated with *S. bovis- equinus* complex infections contribute to 50% percent of mortalities in Kachemak Bay, AK. This condition has only been reported once before in the literature but has now begun to be observed in otters from other areas of Alaska. The high incidence of this infectious cause of death in sea otters is unprecedented. It is our hope that further understanding of this particular pathogen and its pathogenesis will assist in the management of wild populations and help to develop tools to assess the prevalence and impact of this condition throughout the range of the sea otter in the Pacific. If there is evidence that the source of the infection might be anthropogenic, further investigation and potentially mitigation would be indicated.

4) If you received previous CEF funding for this or a related project, please summarize relevant findings and how they relate to the current proposal.

N/A. Previous CEF has not been received for this or a related project.

5) Who are the participants in this project, what are their roles, and what are their qualifications?

Carrie Goertz, MS, DVM, has been the primary rehabilitation veterinarian at the Alaska SeaLife Center for the past year and a half and has both cared for live and necropsied dead sea otters with *S. bovis- equinus* and valvular endocarditis. She will coordinate with zoos and aquariums to obtain samples from captive animals. Additionally, she will assist with the sampling of wild caught animals and provide veterinary oversight for anesthesia.

Pam Tuomi, DVM, has been involved with sea otter care since the *Exxon Valdez* oil spill and wrote the chapter on sea otter medicine in the CRC Handbook for Marine Mammal Medicine. She will advise the project and participate in sample collection and anesthesia as needed.

Verena Gill, MS, Wildlife Biologist, has participated in sea otter management and sample collection since 2002. She will help secure an appropriate vessel and crew for the capture trip and oversee, and participate in capturing and processing the animals. Additionally, she will coordinate the collection needs of other researchers.

Angela Doroff, MS, Wildlife Biologist, has participated in sea otter management and sample collection since the *Exxon Valdez* oil spill. She will advise the project and participate in capture and sample collection. Angela and Verena are Co-PI’s on the otter capture permit used in this project.

Barbara Byrne, DVM, PhD, has been a clinical veterinary microbiologist for 12 years and has participated in the diagnosis and investigation of various sea otter diseases since 2003. She will oversee the culture and analysis of the microbiologic samples.

Kathy Burek, DVM, MS, Diplomate ACVP, has been the primary pathologist advising the group investigating the patterns of disease and mortality in northern sea otters in Alaska and will be responsible for the histopathological examination of any external lesions obtained from animals during the capture trip and will assist in the interpretation of results.
The wild animal capture trip will also be supported by additional experienced sea otter capture personnel and a boat crew.

6) Why do you consider the project to be timely and how does it address a current critical need?

Northern sea otters (Enhydra lutris kenyoni) in southwest Alaska are currently experiencing a severe decline. In order to develop strategies to reverse this trend we need to further investigate and understand major causes of disease and mortality such as S. bovis- equinus complex infections and the associated valvular endocarditis. This pathogen may be associated with human activity and further investigation may help determine effective management strategies to reduce the impact to sea otter populations.

7) Explain how you will ensure that you are meeting the goals, objectives, and outcomes of your project. Include how your evaluation plan will measure program effectiveness and apply evaluation data to strengthen the project. Evaluation plans may be quantitative or qualitative.

Success will in part be measured by the number of wild and captive animals processed and by the collection of viable culture samples and supportive biological samples. This will be ensured by using a wild capture protocol and personnel with an established history of successful efforts in other geographic areas of Alaska. Sample collection, shipping and culture methods have been established in the cooperative sea otter mortality study over the past three years. Additionally, we have received positive responses from the facilities holding captive sea otters offering to assist in providing us samples collected during their routine health assessments. The participants will regularly review the results of each sampling effort to determine if protocols need modification. Once sampling and processing is complete, the group will review all data to determine recommendations for future work and, if possible, to make management recommendations to appropriate agencies. Findings from this study will be subject to peer review when submitted for publication in one or more scientific journals.

8) Will this project be sustainable beyond the life of the grant? If so, explain how.

This project will contribute to a continuing cooperative effort known as “Monitoring Mortality and Health Profiles of Sea Otters in Alaska through Stranded Carcasses” to assess disease and causes of mortality in northern sea otters. This research is considered a high priority in view of the recent threatened listing of the SE Alaska population. The mortality study has been funded through a variety of sources including grants from the Oiled Wildlife Care Network, the Minnesota Zoo and joint contributions from the various collaborators including those participating in the currently proposed project. Additional grant funding opportunities are being sought but basic funding for the mortality study through USFWS and the ASLC Sea Otter Research Program has been assured such that, currently, this greater project has no end date.

9) How and when will the information generated by this project be disseminated?

Information will be shared among all project participants including the federal agency responsible for management of the northern sea otter in Alaska. Results of microbial studies for individual otters will be sent to the participating AZA facility providing the samples. All data
and findings will be presented at AZA, AZVA, or IAAAM conferences. Submission of articles to select peer-reviewed publications will also facilitate dissemination of findings.

10) If this is a multi-year project, what is the funding strategy for subsequent years?

Funding is sought for one live capture trip and for sample analysis for one year only. The analysis of samples obtained from that trip and from participating AZA institutions is expected to take less than one year. Additional facets of the larger Monitoring Mortality and Health Profiles of Sea Otters in Alaska project are funded separately.

11) Is this proposal connected to a relevant AZA program or institution? No

12) Are there any collaborating/partner institutions?

This is a highly collaborative project to include AZA zoos and aquariums, state and federal wildlife agencies, and academia to include the following organizations:

- U.S. Fish and Wildlife Service, Marine Mammals Management
- U.C. Davis, School of Veterinary Medicine, Department of Pathology, Microbiology, and Immunology, Vet. Med. Teaching Hospital Microbiology Laboratory
- Alaska Veterinary Pathology Services
- Alaska SeaLife Center
- Seattle Aquarium
- Oregon Coast Aquarium
- Point Defiance Zoo and Aquarium
- John G. Shedd Aquarium

Verbal confirmation of willingness to assist in sample collection for this project has been received from the Vancouver Aquarium but a signed letter could not be processed in time to submit with this application.

13) Have all the necessary permits been obtained for the project?

Yes. This project will operate under USFWS MMPA permit number MA041309-1 issued by the USFWS Division of Management Authority to conduct marine mammal scientific research on sea otters in coastal Alaska [31 August 2004 – 30 August 2009]). Co-PI’s for this project, Angela Doroff and Verena Gill, are listed on this permit.

14) If research animals are to be purchased for this project, indicate the destination of the animal(s) after the completion of the research or provide justifications for euthanasia.

N/A. Research animals do not need to be purchased.
REFERENCES CITED


2006 Conservation Endowment Fund: **Budget**

**BUDGET**

**PREVIOUS CEF SUPPORT**
The PI has not received previous CEF support.

**PROJECT BUDGET**
Total Project Budget: $24,637 + approx personnel ~$24,000 Total Requested from CEF: $19,797

**Other Support – PERSONNEL**

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### Detailed Budget

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### Budget Justification

Funding is only being requested for the capture and health assessment of 35 live otters plus costs of sample collection/shipping and initial culture and identification of bacterial flora from these otters plus an additional 15 northern sea otters currently held in participating AZA facilities.

**Personnel.** A minimum of six experienced people is necessary to safely conduct capture and sampling of wild sea otters. Sedation and sample collection will take place aboard the support vessel by a separate crew including a veterinarian (the project PI or a collaborator). Given the current sea otter population density in Kachemak Bay, field experience has shown that an average of 4 to 6 otters may be captured and sampled per day so the project has budgeted staff for 8 days of capture. Salaries for all study personnel will be supported by their respective home institutions including some from federal grant sources.
Supplies and commodities. Veterinary supplies include drugs and medications necessary for anesthesia of 35 wild otters plus Amies microbial culturettes, needles, syringes and vacutainer blood tubes, sterile cryovials and pipettes for sample aliquoting and archiving, and miscellaneous sampling supplies (isopropyl alcohol, gauze sponges, tape, markers, etc). Microbial samples will be shipped every other day via overnight express to the U.C. Davis School of Veterinary Medicine Microbiology Laboratory. Shipping costs are included for samples from the capture effort plus delivery of samples collected by contributing AZA facilities. A capture skiff will be provided by the project collaborators but expenses will be incurred for fuel and any necessary maintenance or repairs of capture equipment.

Travel and vessel support. Mileage has been included for roundtrip travel by motor vehicle for staff based in Anchorage and in Seward to the capture site in Homer. Food for the project staff will be necessary for the 8 days of field operations, but housing will be provided at the USFWS bunkhouse in Homer. A discounted vessel charter rate has been negotiated for 8 days for a 48 ft support vessel based in Homer and will include use of the vessel skiff and assistance by the vessel's two person crew for skiff operations, food preparation for project staff, and other support as needed. In addition, travel and per diem has been included for a veterinary ultrasound consultant to travel to Homer to conduct echocardiography on wild captured sea otters.

Equipment. No specialized equipment costs are included in this grant as necessary equipment (capture nets, depth sounder, centrifuge, portable ultrasound, anesthetic monitoring and boating safety equipment) is already owned and will be supplied by the collaborators.

Contracts. Laboratory fees have been negotiated on a fee-for-service basis with the U.C. Davis School of Veterinary Medicine Microbiology Laboratory for initial culture, isolation and identification of the bacterial samples (nasal and/or pharyngeal, rectal and/or fecal) submitted from 50 total animals. Complete blood cell counts and serum chemistry profiles for up to 35 wild caught will be performed at a reference laboratory routinely utilized by USFWS. Samples will be archived for future serological monitoring for diseases of concern, and the participants will continue to apply for additional funding to support this aspect of the project.

Indirect Costs. The Alaska SeaLife Center has agreed to waive the usual 27.08% indirect cost rate for this project.