PROPOSAL: EXPANDED MONITORING OF THREATENED CALIFORNIA SEA OTTERS AND THEIR PREY FOR TERRESTRIAL-ORIGIN PARASITIC AND BACTERIAL PATHOGENS

David A. Jessup and Melissa A. Miller

Marine Wildlife Veterinary Care and Research Center

California Department of Fish and Game

1451 Shaffer Road, Santa Cruz, CA 95060

March 25, 2003

PROJECT PROPOSAL SUMMARY

Project Description

The southern sea otter (*Enhydra lutris nereis*) is a federally listed threatened species found only in central California. Previous postmortem examinations of southern sea otters have implicated infectious agents, such as parasites and bacteria, in the death of nearly 40% of freshly dead otters[1, 20]. Our preliminary data suggest that many sea otter deaths are caused by protozoal parasites and bacteria that are spread by fecal contamination of nearshore marine waters by terrestrial animals or humans[10, 13, 14, 15]. Two species of protozoa, *Toxoplasma gondii* and *Sarcocystis neurona* were isolated from sea otters and harbor seals recovered in or near Elkhorn Slough, and died due to protozoal brain infections[13, 14]. Interestingly, both parasites appear to be spread by terrestrial animals known to be present in the vicinity of Elkhorn Slough, namely domestic cats for *Toxoplasma* and opossums for *Sarcocystis*. Collectively these two parasites are a major cause of mortality for threatened California otters. It is interesting to note that both domestic cats and opossums are introduced species to coastal California that have proliferated over the past 100 years. In a recently published study[18], we detected two "hot spots" along the California coast where otters have high levels of *Toxoplasma* infection. These high-risk areas were centered on the areas of Elkhorn Slough/Moss Landing and Morro Bay, California. Otters sampled at these sites were more likely to be infected by *Toxoplasma* than at all other locations along the central California coast. We also detected an association between exposure to major sources of freshwater runoff and *Toxoplasma* infection in sea otters[18].

In a related study, we determined that 5 species of bacteria (*Campylobacter* spp., *Clostridium perfringens*, *Plesiomonas shigelloides*, *Salmonella* spp. and *Vibrio* spp., including *V. cholera)* and 2 species of protozoan parasites, *Cryptosporidium* and *Giardia* sp. that are known to be shed in the feces of infected terrestrial animals and humans were present in California sea otter feces[15]. For 25% of the fresh dead otters examined during the course of this study, these fecal pathogens may have been a factor in the otter's death. We also determined that 35% of the otters examined were positive for more than one of the pathogens[15]. The scope of this initial study was limited due to funding constraints. However, the presence of these pathogenic bacteria and parasites in California sea otters suggests that terrestrial animal and human waste may be having an adverse impact on nearshore ecosystems in California. As nearshore-dwelling marine mammals that feed on large volumes of potentially contaminated, filter-feeding marine invertebrates, sea otters serve as excellent marine water quality sentinels. Once present in the nearshore environment, these pathogens may negatively impact sensitive marine species such as sea otters, marine ecosystems, human coastal recreational opportunities, and could even impact human health. We believe our preliminary findings have significant conservation, water management and public health implications and merit more in-depth investigation.

To address this emerging problem of nearshore biological pollution, we propose a series of inter-related studies that will provide management agencies with more detailed information on the scope and nature of terrestrial-origin biological pollution in central California's nearshore marine environment. These studies are interconnected and complementary, and will address in detail the detection and landward source identification of suspected biological pollutants. The proposed studies will focus on the two highest risk areas: Elkhorn Slough/ Moss Landing and Morro Bay, but because of the broad focus of our collaborative research team, we will also be able address these environmental issues on a sea otter range-wide basis. We will test both sea otters and their filter-feeding invertebrate prey for exposure to specific species of pathogenic parasites and bacteria, so that a more comprehensive picture of the flow of these biological

pollutants from the land to the sea may begin to emerge. These studies will include: 1) Expanded testing of sea otter tissues and feces for selected species of protozoal and bacterial pathogens (listed below). Sea otters sampled from a relatively pristine area along the Big Sur coast will be used for comparison. 2) Screening of sea otter invertebrate prey, including filter-feeding bivalves and crustaceans, for uptake of these same parasites and bacteria, thus potentially serving as a source of infection for sea otters and/ or humans. 3) Molecular (DNA) and/ or antigenic characterization of selected isolates from sea otters and invertebrates to help identify potential sources of these pollutants. Where possible we will use advanced diagnostic techniques to compare isolates obtained from otters and invertebrates with those derived from humans or terrestrial animals. 4) Test serum from live and dead otters sampled near the two high-risk areas, as well as a control area for the presence and concentration (titer) of antibodies to *Toxoplasma* and *Sarcocystis neurona*. 5) Compile our results so as to highlight important associations and risk factors for pathogen exposure that will be of management significance to state and federal resource agencies. This research will be done in collaboration with our colleagues at the University of California-Davis, the Central Coast Water Quality Control Board, and the United States Geological Survey, and will greatly contribute to our understanding of potential links between human and terrestrial animal waste discharge, urban runoff, disease in southern sea otters and biological contamination of their prey at selected locations along the California coast. We also plan to coordinate our research with other, ongoing or proposed research for the Elkhorn Slough and Morro Bay regions as well as the region wide efforts of the Regional Board's Central Coast Ambient Monitoring Program (CCAMP).

Cost, Administration and Reporting

We propose a budget of $240,000, to be utilized over two years to complete the above research objectives. The funds will be administered by the University of California-Davis, Wildlife Health Center to support work done by our collaborators at U.C. Davis School of Veterinary Medicine and at our own CDFG facility, the Marine Wildlife Veterinary Care and Research Center in Santa Cruz. CDFG will provide the Regional Water Board with an interim report one year following funding and a final report 3 months after the end of the project. Several scientific publications are expected to result from this work.

PROJECT PROPOSAL

Introduction

The southern sea otter (*Enhydra lutris nereis*) is a federally listed threatened species, found only in central California. The current southern sea otter population is less than 2400 individuals. Examination of our recent sea otter postmortem data revealed that the proportion of freshly dead sea otters dying of infectious disease has increased, from 38.5% for otters examined between 1992 and 1996, to over 45% for otters examined between 1998 and 2001[10]. Our data suggest that some fatal infections in California sea otters and harbor seals are caused by single-celled parasites (protozoa) and bacteria that are also pathogenic to humans, and may emanate from the fecal wastes of terrestrial animals and humans[10,13,14,15]. In a pilot study funded by the Central Coast Regional Water Board via settlement monies provided by the City of Pacific Grove, and in related followup work, we isolated *Campylobacter* species, *Clostridium perfringens*, *Plesiomonas shigelloides*, *Salmonella* spp. and *Vibrio* spp., including *V. cholera,* from sea otter feces, and also detected gastrointestinal infections by the protozoan parasites *Cryptosporidium* and *Giardia*[15]*.* For this study, fecal samples were collected from apparently healthy, wild sea otters sampled for field research projects (15%), from known otter haul-out sites (65%), and from live-stranded and fresh dead sea otters (20%). It is interesting that for 25% of the fresh dead otters, the organisms may have played a role in the death of the animal. In addition, all but one of the potential fecal pathogens we tested for (*Escherichia coli* 0157:H7) were present in sea otter feces, and multiple intestinal pathogens were isolated from 35% of the otters.

In addition to being commonly isolated from California sea otters, some of the above pathogens appear to be associated with significant sea otter mortality. In a study that is currently in press, we determined that the protozoan parasite *Toxoplasma gondii* was the primary cause of death for 16.2% of southern sea otters examined at our facility from 1998 to 2001, and contributed to the death of an additional 11.4% of otters[10]. Cat feces are the only currently identified definitive source of the infective oocyst, or “egg” stage of *Toxoplasma*, and millions of these environmentally-resistant eggs may be shed in the feces of an infected cat. A second protozoal parasite *(Sarcocystis neurona)* is also a significant cause of mortality in sea otters. This parasite has a similar life cycle to *Toxoplasma*, except that opossums serve as the definitive, or egg-shedding hosts. *Sarcocystis*-associated brain disease was the primary cause of death for 6.7% of sea otters examined from 1998 to 2001, and contributed to the death of an additional 5.7% of otters[10]. Together these parasites caused death of 29% of California sea otters examined from 1998 to 2001 and contributed to the death of another 18% of otters[10]. 0verall, over 4% of the 105 freshly dead otters necropsied between 1998 and 2001 had bacterial infections as a primary or contributing cause of mortality. Bacteria implicated in these fatal infections in otters included recognized fecal pathogens such as *E. coli*, *Salmonella* sp. and *Vibrio parahemolyticus*. Collectively, over 45% of necropsied otters enrolled in this study had infectious agents (bacteria, parasites and fungi) as a primary or contributing cause of death. We believe these findings may be significant at the population level, and may be one factor in the recent declines observed in the California sea otter population.

In a recently published study, we found that otters exposed to heavy freshwater runoff were almost three times more likely to be infected with *Toxoplasma*[18]. In addition, two high-risk areas for *Toxoplasma* infection were identified along the central California coast. These “hot spots” were centered on Elkhorn Slough/Moss Landing and Morro Bay, California. Both locations share some common factors that might be contributing to this elevated risk. Both are

estuarine environments with reduced flushing compared to open coastline environments. Both have known problems from fecal contamination from agricultural, urban or other sources; both are listed as impaired by pathogens on the CWA 303(d) list of impaired waterbodies. Both areas are associated with significant numbers of outdoor cats.

In addition to being "high-risk" areas for *Toxoplasma* exposure as outlined above, both Elkhorn Slough/ Moss Landing and Morro Bay also appear to be high-risk areas for otter mortality due to infection by protozoa and bacteria. In a study in press, we determined that the Morro Bay region especially was a "hot spot" for *T. gondii*-related mortality for sea otters[18]. Both areas experience both seasonal and periodic fluctuations in sea otter populations. As many as 65 otters have been observed within Elkhorn Slough in the recent past. The average population within the Slough itself is more typically about 20 otters at the present time, with many additional otters utilizing Elkhorn Slough, Moss Landing and the immediate vicinity both transiently and seasonally. Based on our records, between 1998 and 2002 over 30 freshly dead California sea otters were found within 20 km of Elkhorn Slough, and numerous additional autolyzed (decomposed) sea otter carcasses were recovered from this same area during the same time period. For the 30 fresh dead otters that were found dead in the immediate vicinity of Elkhorn Slough/ Moss Landing between 1998 and 2002, brain inflammation caused by *Toxoplasma* or *Sarcocystis neurona* were suspected as a primary or contributing cause of death for 7 otters (23%), and bacterial infections were implicated as a primary or contributing cause of death for 7 otters (23%). Other significant causes of otter mortality in the vicinity of Elkhorn Slough/ Moss Landing included peritonitis due to acanthocephalan (thorny-headed) worms (n = 6), white shark predation (n = 5) and boat strike (n = 2). The pathogenic protozoans and bacteria isolated from otters from the vicinity of Elkhorn Slough included *Toxoplasma gondii*, *Sarcocystis neurona*, *Salmonella* sp., *Clostridium perfringens* type A, *Vibrio* sp. and *Plesiomonas shigelloides*.

Similarly, at least 33 freshly dead otters stranded within 20 km of Morro Bay between 1998 and 2002. For these 33 otters, protozoal brain infections caused by *Toxoplasma* or *Sarcocystis neurona* were suspected as a primary or contributing cause of death for 12 of the otters (36%), and bacterial infections were implicated as a primary or contributing cause of death for 7 otters, or 21%. Other significant causes of otter mortality in the vicinity of Morro Bay included peritonitis due to acanthocephalan (thorny-headed) worms (n = 6), white shark predation (n = 3) and boat strike (n = 2). The pathogenic protozoans and bacteria isolated from otters from the vicinity of Morro Bay included *Toxoplasma gondii*, *Sarcocystis neurona*, *Cryptosporidium* sp., *Enterococcus* sp., *Clostridium perfringens* type A, *Vibrio parahemolyticus* and *Plesiomonas shigelloides*.

The specific source(s) of these pathogens for sea otters are currently unknown, but we believe that some protozoal and bacterial fecal pathogens detected in sea otters could be of human or terrestrial animal origin, and may be entering the marine environment via surface runoff and/ or sewage effluent. Recent studies have confirmed that shellfish, such as mussels, clams and oysters are capable of removing the "eggs" of *Toxoplasma*, *Cryptosporidium* and *Giardia* from contaminated water and concentrating them in their tissues[2,3,4,5,7,8,11]. Once present in the shellfish tissues, these parasites may remain viable for long periods of time, and may serve as a source of infection for otters or humans when the shellfish are consumed. Sea otters lack a blubber layer and must consume up to 25% of their own body weight in invertebrates each day to maintain their body weight. Thus, biological contamination of filter-feeding invertebrates such as shellfish and crabs by protozoa and bacteria could prove to be an efficient route for infection of sea otters, and could help explain the high proportions of *Toxoplasma*-infected otters that we have detected in California. Because of their nearshore habit and

reliance on filter-feeding prey as a primary food source, we feel that our findings to date in sea otters may be indicative of a bigger, and as-yet-unrecognized environmental and human health problem that merits detailed investigation. If we confirm that these pathogenic protozoa and bacteria are being removed from contaminated water and transmitted to sea otter by filter-feeding prey, this study could have important human health significance, because sea otters and humans consume some of the same nearshore invertebrates, such as clams, mussels and crabs, and several parasites and bacteria that we are detecting in sea otters can also cause disease in humans.

Materials and Methods

a. Otter feces

Fresh feces will be obtained from at least 60 sea otters over a 2 year period and screened for the presence of the following bacteria: *Campylobacter* sp., *Clostridium perfringens*, *Clostridium difficile*, *Escherichia coli* 0157:H7, *Plesiomonas shigelloides*, *Salmonella* sp. and *Vibrio* sp. In addition we will screen the feces for the protozoan parasites *Giardia and Cryptosporidium*, with molecular characterization of isolates. Submitted fecal samples will be divided between 3 different geographic locations: Elkhorn Slough/ Moss Landing (n = 20), Morro Bay (N = 20) and, as a control, the more pristine and less developed Big Sur Coast (n = 20). The sample sites will encompass sampled otters or known haulout locations within the immediate vicinity of each sample location (±20 km), except for the Big Sur coast, which will include data for all otters sampled within this remote and relatively inaccessible region. Fecal samples will be collected from live and freshly dead sea otters for this study. Samples from live-captured otters will be collected from previously disinfected transport kennels during capture operations. Feces of freshly dead sea otters will be obtained from the lower intestines during necropsy (postmortem examination). Otters which had been treated with antibiotics prior to death will be excluded from this study. The remaining samples will be obtained by collecting scat (feces) opportunistically from free-living otters at known haul-out (resting) sites. Each fecal sample will be placed in a sterile container, refrigerated, and shipped by overnight mail to the Microbiology Laboratory at the Veterinary Medical Teaching Hospital, Davis, California for bacterial culture, fecal floatation, immunofluorescence, immunomagnetic bead separation (IMS) and, for selected isolates, PCR or fatty acids analysis. Each fecal sample will be screened for the presence of the protozoa *Cryptosporidium* and *Giardia* using fecal floatation, direct immunofluorescent antibody testing (DFAT) and/ or IMS. For the fecal floatation test, a solution with a high specific gravity is mixed with the fecal sample, which causes the parasite eggs to float at the surface of the mixture, so they can be collected on a glass slide and observed on a microscope. The DFAT test utilizes monoclonal antibodies labeled with fluorescent markers to indicate the presence of these protozoal parasites when the sample is observed with a microscope. For parasite detection using immunomagnetic beads, the sample is mixed with magnetic beads coated with monoclonal antibody, allowing the parasites to be concentrated while getting rid of other particulate debris. Samples testing positive via the above tests will be preserved for more precise DNA testing being conducted as another part of this project.

Bacterial cultures will be performed on each fecal sample using direct culture techniques, selective media and standardized methodology[9,12,19,21]. When required, bacterial identification will be confirmed using additional tests, including fatty acids analysis, serotyping and/or DNA amplification and sequencing of key bacterial genes. All *E. coli* isolates obtained from feces will be screened for antigenic epitopes consistent with the recognized human pathogenic strain

designated 0157:H7. *Salmonella* isolates will be submitted to the National Animal Disease Laboratory (NADL) for confirmatory serotyping. *Campylobacter* sp. isolates will be confirmed by phenotypic characteristics. Isolates of *Clostridium perfringens* will be tested for production of *Clostridium perfringens* enterotoxin (CPE) using commercially available kits or PCR. Significant growth of bacteria other than those listed above will be noted, including *Enterococcus* sp., *Edwardsiella tarda* and *Aeromonas* sp. Our preliminary study on this topic[15] yielded numerous sea otter fecal samples that were positive for the above pathogens, but was limited by small sample size (n = 40) due to funding constraints. We have worked to increase the sample size for this ongoing study, but have been limited by lack of funds. However, approximately 20 additional animals were recently sampled, bringing our current total to 60 otters. We propose to pool data from the proposed study with the existing body of data from this previous study to increase our sample size and to enable us to address specific questions regarding spatial clustering of otters testing positive for these selected parasites and bacteria along the coast. In the case of otters sampled at necropsy, increasing our sample size may also allow us to better examine the statistical associations between pathogen isolation from feces and evidence of disease in otters. In addition, it will allow for banking of protozoal and bacterial isolates from sea otters to facilitate future molecular comparisons with similar organisms isolated from humans and terrestrial animals with gastrointestinal disease. At the completion of this 2 year study we will have screened feces for the above-listed pathogenic protozoa and bacteria from at least 120 California sea otters.

b. Otter tissues

Our facility examines all of the 150-200 California sea otters found dead along the California coast each year. During the proposed two year study, all otters found freshly dead (approximately 50 otters per year) will receive a complete postmortem examination, including full-body radiographs and examination of tissues on a compound microscope. Bacterial culture will be completed for any abnormalities observed during the examination, and feces will be submitted for bacterial culture from any otters that had not received antibiotics prior to death. Necropsied otters will be tested for the presence of the above-listed fecal bacterial and protozoal pathogens using a combination of techniques, including culture on agar media or cells, immunofluorescence, PCR, serotyping or fatty acids analysis, as outlined above. Sea otter brain tissue will be collected aseptically at the time of postmortem examination, placed in sterile saline solution and shipped refrigerated by overnight mail to Dr Patricia Conrad's laboratory at the School of Veterinary Medicine at U. C. Davis. At Davis, the sea otter brain tissue will be homogenized, layered over cells in culture and incubated at 37°C for two hours. During this time, any *Toxoplasma* or *Sarcocystis neurona* parasites present in the brain tissue can enter and infect the live, cultured cells. Tissue-exposed cell cultures will be held at 37° C for up to 30 days, and the cultures will be fed and inspected twice weekly for evidence of infection using an inverted microscope. The identity of parasite isolates obtained using this technique will be confirmed using restriction fragment length polymorphisms (RFLP) and/ or DNA amplification and sequencing, and isolates will be banked for later comparative studies.

c. Otter serum

All live and dead sea otters sampled during the course of this study will have their geographical location at the time of stranding or live-sampling recorded to the nearest 0.5 km, and will be screened for the presence and concentration (titer) of serum antibodies to *Toxoplasma gondii* and *Sarcocystis neurona* using immunofluorescent antibody tests (IFAT) previously developed for sea otters by Drs Miller, Conrad and colleagues[16]. We have validated the sea otter *T. gondii* IFAT, using serum from 77 necropsied otters with confirmed *T. gondii*

infection status[16]. Validation of the IFAT for *S. neurona* will be completed when a sufficient number of positive control samples are obtained. Live sea otters will be sampled as part of ongoing fieldwork on sea otter biology, foraging and long-term survival. We anticipate that serum from at least 100 live and 100 dead otters will be sampled during the course of this two year study. In addition, we maintain an extensive bank of over 500 cryopreserved serum samples at our facility dating from 1990 to the present, and can select additional banked serum samples if needed for testing to allow us to obtain adequate statistical power to address the specific spatial, temporal or environmental questions in relation to our study objectives. The precise otter sampling locations will be determined by the location of dead-stranded otters, live study animals and the objectives of the ongoing fieldwork to some extent, but will be correlated with our high and low-risk study sites and invertebrate sampling sites to the best possible degree. Because our collaborative sea otter work encompasses the entire sea otter range (from Half Moon Bay to Santa Barbara), we will also consider areas outside of the previously-determined main focus areas in this comprehensive study, so that larger environmental questions may also be addressed.

d. Site selection and testing of invertebrate prey

Our proposed research will focus on two locations along the California coast that have been determined in previous studies to be high-risk areas for terrestrial pathogen exposure for sea otters, including the human pathogenic parasite *Toxoplasma*[18]*.* Our preliminary data suggests that possible terrestrial-origin bacterial fecal pathogens are also present in sea otters at both locations (please see Introduction for details): One high-risk site is centered on Elkhorn Slough/ Moss Landing, and a second, replicate high-risk site is centered on Morro Bay. The purpose for including both sites in the study is to provide comparative data for these two “hot spots”, as well as to enhance our ability to detect patterns for land-sea contamination. A more pristine location along the Big Sur coast will serve as a control site for comparison of ecological risk factors, such as proximity to known sources of surface runoff, municipal sewage or dense human populations. Since we currently examine and sample sea otters from throughout their California range (Half Moon Bay to Santa Barbara), we will be able to provide additional background data on otters sampled from outside of the main study areas, and could include additional sampling locations if a potential biological pollution problem is detected during the course of this study.

During the first year of the study, resident or explanted wild native mussels (*Mytilus* spp.) (n = 30/ site) and/ or other invertebrate prey, such as sand crabs (*Emerita analoga*) (n = 30/ site, where present) and/ or fat innkeeper worms (*Urechis caupo*) (n = 30/ site, where present) will be sampled twice each year from the vicinity of Elkhorn Slough and its replicate high-risk site, Morro Bay, to coincide with the heavy rainfall periods and the summer dry season. At least 4 subsites within each location (Morro Bay or Elkhorn Slough) will be selected for sampling. Thus at least 120 invertebrates will be sampled at each site during each sampling period, and over 240 invertebrates of each of 3 species (eg. *Mytilus* sp. *Urechis caupo* and *Emerita analoga*) will be sampled in total at each high-risk location (Elkhorn Slough/ Moss Landing and Morro Bay) during the course of this study. A pristine site along the Big Sur coast will also be sampled as a control. Explanted native invertebrates will be held in filtered seawater to allow for depuration, and will be sub-sampled using PCR analysis on hemolymph to confirm negative protozoal infection status prior to placement. Clean, depurated native mussels will be placed at locations within each high-risk area: Elkhorn Slough/ Moss Landing and Morro Bay. We will use molecular techniques (polymerase chain reaction, or PCR) to test invertebrate tissues for the presence of the protozoan parasites *Toxoplasma* and *Cryptosporidium.* In addition, a subset of these invertebrates (n = 30/ site, or approximately 10 of each of the three species) will be sampled

and cultured twice each year for the same bacterial pathogens as the sea otter feces and tissues, including *Campylobacter*, *Clostridium perfringens*, *Clostridium difficile*, *Escherichia coli* 0157:H7, *Plesiomonas shigelloides*, *Salmonella* sp. and *Vibrio* spp. Invertebrate placement and collection sites will be selected so as to be maximally exposed to freshwater runoff, agricultural runoff, agricultural waste, municipal sewage or urban runoff. Invertebrate samples will be collected and tested for the presence of the above-listed bacterial and protozoal pathogens using a combination of techniques, including culture on agar media or cells, immunofluorescence, PCR, serotyping or fatty acids analysis, as outlined above. Isolates will be banked for later studies to more precisely compare them with similar isolates obtained from sea otters, terrestrial animals and humans.

e. Data Analysis

The resulting data from otter fecal and serum analysis, postmortem examinations and invertebrate testing will be analyzed for associations between infection by the above-listed pathogens and defined spatial and environmental risk factors. In this respect, the proposed study will be similar to a project that we completed last year[18]. For this previous study, the location of all live or dead sea otters examined by our facility between 1997 and 2001 was recorded to the nearest 0.5 km, and the data was used to examine coastal risk factors for *Toxoplasma* infection in California sea otters[18]. Specifically, we examined the relationship between seropositivity to *Toxoplasma* in otters and exposure to three major categories of risk factors, including demographic risk factors (such as otter age or gender), environmental risk factors (such as exposure to runoff or sewage), and spatial risk factors such as otter location at the time of sampling[18]. Data on locations of major sources of municipal sewage and major streams and rivers were obtained through collaborations with the Central Coast Regional Water Quality Control Board, and these collaborations will continue for the proposed study. Data on human coastal population density was obtained from 2000 census data. The sensitivity, specificity and cut-offs for otter serum testing for *Toxoplasma* were previously established through test validation[16].

For the proposed study we will take the next step along this path of investigation: We will begin the process of tracing the path of potential biological pollutants from the sea, back to the land in greater detail, and will focus our attention on two coastal areas (Elkhorn Slough/ Moss Landing and Morro Bay) that were identified as high-risk sites during the previous study. Both high-risk sites share some common attributes, particularly being centered on large enclosed bays or sloughs. For the proposed study, we will monitor otters from both high-risk areas, and test filter-feeding prey from the same areas for the presence of pathogenic protozoa and bacteria. Sites for collection of otter prey to be monitored within each area will be selected with the location of potential plumes of polluted water in mind. Other factors that will be considered include the abundance and distribution of these invertebrate prey. Unless limited by site restrictions, a minimum of 30 invertebrates will be collected at each of 4 sites on a twice yearly basis, with sampling times optimized to sample during periods of maximal and minimal rainfall. The selected sample size of 30 invertebrates per sample site and sample period is based on the assumption that the prevalence of pathogenic protozoans in invertebrates within the high-risk areas is at least 10%, and that we want 95% confidence to detect at least one positive sample per group. Comparison samples of invertebrates will also be collected from a low-risk site along the Big Sur coast to verify that they have a low prevalence of parasite infection. For initial hypothesis testing, we will utilize chi-square analyses to examine the associations between pathogen isolation and categorical variables, such as high-risk or low-risk status, while t-tests will be used to evaluate the association with continuous variables, such as number of days since the last rainfall. Once univariate analyses are completed to ascertain statistical patterns, logistic

regression models will be constructed to allow us to evaluate these relationships in more precise detail, while accounting for confounding factors, interactions and sample bias. Data from our replicate high-risk sites (Elkhorn Slough/ Moss Landing and Morro Bay) will be examined separately and together, and will be compared to a third, lower-risk site (the Big Sur coast). This control site has a low coastal human population density, few large streams or rivers and minimal to no discharges of municipal sewage or urban runoff. The spatial software program SaTScan will be used to examine the data for spatial clustering of selected protozoal or bacterial isolates along the coast. Using detailed data on coastal land use and wastewater discharges that were previously obtained from the Central Coast Water Quality Control Board, as well as our locations for shellfish and sea otter sampling, we will also examine patterns of isolation for bacteria and protozoa with respect to recognized sources of freshwater or agricultural runoff, sewage, urban runoff and other potential sources of contamination. Selected isolates will also be characterized using biochemical and molecular techniques, and compared with similar isolates obtained from humans and terrestrial animals to seek patterns of exposure that could provide important clues as to the sources and pathways of nearshore marine contamination.

Other Project Features

Over the past 5 years we have developed an excellent working relationship with the U.C. Davis School of Veterinary Medicine. As a result we are closely collaborating with their faculty, graduate students and staff to address complex biological questions in a cost and time-efficient manner. We will continue these highly productive collaborations for the proposed project. As a side benefit of this collaboration, the U.C. Davis group that we work with operates with relatively low administrative charges. Thus, the project funding can have maximal effectiveness in addressing the proposed environmental questions with minimal diversion of the funds into administrative costs. Our collaborative group is sensitive to the concerns of potential stakeholders in this research. In our previous study of fecal contamination, we identified locations in the final report using only general descriptions, and made specific locations of these isolations known only on an as-needed basis, recognizing that the data was preliminary. To address local sensitivity and to increase project collaboration, we propose to do the same in this current study. We also recognize the importance of conveying our research results back to the agencies tasked with maintenance of coastal water quality in a time-efficient manor. To that end, we will provide our collaborators at the Central Coast Water Quality Control Board with yearly progress reports and a comprehensive final report.

Summary

Our proposed project will utilize advanced diagnostic techniques to explore the potential land-sea connection in the flow of biological pollutants, with a focus on two previously established “hot spots” for sea otter exposure at Elkhorn Slough and Morro Bay. We will screen feces, serum and tissues from a larger number of sea otters and over a longer time period, will encompass a wider diversity of habitats. We will also incorporate a close look at their invertebrate prey in the same habitats. Through this broad approach we can develop a much better understanding of the patterns of exposure, and ways that these infections could be prevented. Throughout the study period we will continue to complete detailed postmortem examinations on all dead sea otters that are recovered and will make that information available to maximize the scientific impact of these studies. Our integrated approach (incorporating a top marine carnivore and its prey in the same comprehensive study) has high potential to yield

information that could be critical for nearshore water quality maintenance, wildlife health, human recreational use of nearshore waters and human health. Molecular characterization of selected isolates will allow us to examine our data for potential connections that might exist between terrestrial animals and humans and nearshore marine wildlife. The end product of this important research will be solid scientific data. That data will enable the various stakeholders, including the Central Coast Regional Water Quality Control Board and CDFG's marine pollution personnel to make intelligent, practical management decisions, particularly for the high-risk areas centered on Elkhorn Slough/Moss Landing and Morro Bay.

Budget and Administration

	Yearly cost	Total Cost
1. Personnel costs (including salary and benefits)	$ 82,100	$164,200
a. CDFG Santa Cruz	($ 51,100)	($102,200)
b. UC Davis School of Vet. Med.	($ 31,000)	($ 62,000)
2. Laboratory costs	$ 26,000	$ 52,000
3. Travel and per diem	$ 750	$ 1,500
4. Publication and reports	$ 250	$ 500
5. Administration and overhead (10%)		$ 21,820
TOTAL COST:		$240,020

References

1. Cole, R. A., D. S. Lindsay, C. L. Roderick, J. P. Dubey, N. J. Thomas, and L. A. Baeten. 2000. Biological and molecular characterizations of *Toxoplasma gondii* obtained from southern sea otters (*Enhydra lutris nereis*). J. Parasitol. 86: 526-530.

2. Fayer, R., T. K. Graczyk, E. J. Lewis, J. M. Trout, and C. A. Farley. 1998. Survival of infectious *Cryptosporidium parvum* oocysts in seawater and eastern oysters (*Crassostrea virginica*) in the Chesapeake Bay. Appl. and Envir. Microbiol. 64: 1070-1074.

3. Fayer, R., E. J. Lewis, J. M. Trout, T. K. Graczyk, M. C. Jenkins, J. Higgins, L. Xiao, and A. A. Lal. 1999. *Cryptosporidium parvum* in oysters from commercial harvesting sites in the Chesapeake Bay. Emerg. Infect. Dis. 5: 706-710.

4. Graczyk, T. K., C. A. Farley, R. Fayer, E. J. Lewis, and J. M. Trout. 1998a. Detection of *Cryptosporidium* oocysts and *Giardia* cysts in the tissues of eastern oysters (*Crassostrea virginica*) carrying principal oyster infectious diseases. J. Parasitol. 84: 1039-1042.

5. Graczyk, T. K., R. Fayer, M. R. Cranfield, and D. B. Conn. 1998b. Recovery of waterborne *Cryptosporidium parvum* oocysts by freshwater benthic clams (*Corbicula fluminea*). Appl. and Envir. Microbiol. 64: 427-430.

6. Graczyk, T. K., Y. R. Ortega, and D. B. Conn. 1998c. Recovery of waterborne oocysts of *Cyclospora cayetanensis* by Asian freshwater clams (*Corbicula fluminea*). Am.J.Trop.Med.Hyg. 59: 928-932.

7. Graczyk, T. K., R. Fayer, D. B. Conn, and E. J. Lewis. 1999a. Evaluation of the recovery of waterborne *Giardia* cysts by freshwater clams and cyst detection in clam tissue. Parasitol. Res. 85: 30-34.

8. Graczyk, T. K., R. Fayer, E. J. Lewis, J. M. Trout, and C. A. Farley. 1999b. *Cryptosporidium* oocysts in Bent Mussels (*Ischadium recurvum*) in the Chesapeake Bay. Parasitol. Res. 85: 518-521.

9. Hirsh, D. C., and Y.C. Zee. 1999. Veterinary Microbiology, 1st ed. Blackwell Science, Inc., Malden, Massachusetts, 479 p.

10. Kreuder C, M. A. Miller, D.A. Jessup, M.D. Harris, J.A. Ames, T.E. Carpenter, J.A.K. Mazet, 2002. Emerging causes of mortality in southern sea otters. In press: Journal of Wildlife Disease.

11. Lindsay, D. L., K. K. Phelps, S. A. Smith, G. Flick and J. P. Dubey. 2001. Removal of *Toxoplasma gondii* oocysts from sea water by Eastern Oysters (*Crassostrea virginica*). Journal of Eukaryotic Microbiology. 197S-198S.

12. Merchant, I.A. and R.A. Packer. 1967. Veterinary Bacteriology and Virology, 7th ed. Iowa State University Press, Ames, Iowa, 752 p.

13. Miller, M. A., P. R. Crosbie, K. Sverlow, K. Hanni, B.C. Barr, N. Kock, M. J. Murray, L. J. Lowenstine, and P.A. Conrad. 2001. Isolation and characterization of *Sarcocystis* from brain tissue of a free-living southern sea otter (*Enhydra lutris nereis*) with fatal meningoencephalitis. Parasitology Research 87: 252-257.

14. Miller, M. A., K. Sverlow, P. R. Crosbie, B. C. Barr, L. J. Lowenstine, F. M. Gulland, A. Packham, and P.A. Conrad. 2001. Isolation and characterization of protozoal parasites from a pacific harbor seal *(Phoca vitulina richardsi*) with meningoencephalitis. Journal of Parasitology 87(4): 816-22.

15. Miller M, N. Christian, E. Dodd, M. Murray, D. Jessup. 2001. Monitoring threatened southern sea otters for exposure to selected fecal pathogens. Report to Regional Water Board. 14 pp.

16. Miller, M., I. A. Gardner, A. Packham, J. K. Mazet, K. D. Hanni, D. Jessup, J. Estes, R. Jameson, E. Dodd, B. C. Barr, L. J. Lowenstine, F. M. Gulland and P. A. Conrad. 2002. Evaluation of an indirect fluorescent antibody test (IFAT) for demonstration of antibodies to *Toxoplasma gondii* in the sea otter (*Enhydra lutris*). J. Parasitol. 88: 594-599.

18. Miller, M., I. Gardner, C. Kreuder, D. M. Paradies, K. R.Worcester, D. A. Jessup, E. Dodd, M. D. Harris, J. A. Ames, A. E. Packham and P. A. Conrad. 2002. Coastal Freshwater Runoff is a Risk Factor for *Toxoplasma gondii* Infection of Southern Sea Otters (*Enhydra lutris nereis*). Int. J. Parasitol 32: 997-1006.

19. Murray, P.R., E.J. Baron, M. A. Pfaller, F.C. Tenover, and R.H. Yolken. 1995. Manual of Clinical Microbiology, 6th ed. American Society for Microbiology, Washington, D.C., 1482 pp.

20. Thomas, N. J, and R. A. Cole. 1996. The risk of disease and threats to the wild population. Endangered Species Update Special Issue: Conservation and management of the southern sea otter. 13: 23-27.

21.Thornton, S. M., S. Nolan, and F. M. Gulland. 1998. Bacterial isolates from California sea lions (*Zalophus californianus*), harbor seals (*Phoca vitulina*), and northern elephant seals (*Mirounga angustirostris*) admitted to a rehabilitation center along the central California coast, 1994-1995. Journal of Zoo and Wildlife Medicine 29(2): 171-176.