CALIFORNIA OCEAN PROTECTION COUNCIL

Staff Recommendation
June 24, 2010

IMPROVED DETECTION OF *TOXOPLASMA GONDII*

File No.: 08-165
Project Manager: Neal Fishman

RECOMMENDED ACTION: Authorize the disbursement of an amount not to exceed $380,706 to the Regents of the University of California, U.C. Davis School of Veterinary Medicine, to research, develop and validate protocols to detect a fecal parasite, *Toxoplasma gondii*, in water samples.

LOCATION: Project outcomes will initially be applied in coastal California. The technology will also have application statewide and nationally.

STRATEGIC PLAN OBJECTIVE: Ocean and Coastal Water Quality, Research and Monitoring

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EXHIBITS

Exhibit 1: Map of Southern Sea Otter Locations

Exhibit 2: Letter of Support

RESOLUTION AND FINDINGS:

Staff recommends that the Ocean Protection Council adopt the following resolution pursuant to Sections 35500, *et seq.* of the Public Resources Code:

"The Ocean Protection Council hereby approves the disbursement of an amount not to exceed $380,706 to the Regents of the University of California for work by U.C. Davis School of Veterinary Medicine to research, develop and validate protocols to detect the parasite *Toxoplasma gondii* in marine and fresh water samples."

Staff further recommends that the Council adopt the following findings:

"Based on the accompanying staff report and attached exhibits, the Council hereby finds that:

1. The proposed project is consistent with the purposes of Division 26.5 of the Public Resources Code, the Ocean Protection Act.
2. The proposed project is consistent with the Ocean Protection Council's grant program funding guidelines."

PROJECT SUMMARY:

The proposed project seeks to develop standardized water testing methods to detect the water-borne parasite *Toxoplasma gondii*, similar to the current U.S. Environmental Protection Agency (EPA) 1623 protocols for detecting *Giardia* and *Cryptosporidium*. The parasite causes a disease called toxoplasmosis which can lead to birth defects and neurologic disease in humans and can cause a brain disease, resulting in mortality in southern sea otters, a federally listed threatened species. Infection by *Toxoplasma gondii* can occur as a result of drinking contaminated water, eating infected and undercooked meat, or through transplacental transmission from mother to fetus. Development of a practical water testing method to detect the parasite will assist in the recovery of the threatened sea otter, as well as improve water quality monitoring and public health for humans.

Improvement of ocean and coastal water quality, and water quality monitoring in particular, is a goal of the OPC Five Year Strategic Plan.

PROJECT DESCRIPTION:

Project Background

Fecal pathogen pollution in aquatic ecosystems is an ever-increasing concern in California as it poses a threat to human and animal health. Potential sources of pollution include agricultural runoff containing livestock feces, sewage-system spills, leaks and outfalls containing human and pet feces, and storm runoff containing feces of domestic and wild animals. Water quality monitoring and control measures are of increasing importance as mounting evidence shows that human communities and their associated animals are having a significant negative impact on the health of coastal ecosystems through impaired water quality. With over half of the state's population living in coastal counties, improving water quality is also a significant public health concern.

Prevalence and Effects of Toxoplasmosis

Outbreaks of toxoplasmosis, the disease caused by *Toxoplasma gondii*, have been reported worldwide and are a cause of significant concern for public health, with up to a third of the global human population infected, including 15-40% of humans in the United States[1]. *T. gondii* infection often goes undiagnosed in humans since most people demonstrate no symptoms or only a mild flu-like illness initially. After the first few weeks of infection have passed, the parasite rarely causes any symptoms in otherwise healthy adults. However, *T. gondii* can cause fatal encephalitis in immunosuppressed people and can cause congenital disease and even death in infants when women are infected during pregnancy. Recent research has also shown a link between *T. gondii* infections and neurologic or behavior changes, such as schizophrenia, in chronically infected individuals.

In addition, the waterborne parasite has increasingly been found to be the cause of fatal brain disease in southern sea otters, a federally listed threatened species, and is now considered an important contributor to southern sea otter mortality in coastal California. Previous studies

[1] J.P. Dubey, 2004. "Toxoplasmosis – a waterborne zoonosis." Veterinary Parasitology, 126:57–72.

conducted by researchers at the U.C. Davis School of Veterinary Medicine have found that approximately half of the southern sea otter population has been exposed to the waterborne pathogen, and that a third of all southern sea otter deaths are due primarily or in-part to *T. gondii* infection.[2] The near-shore distribution and lifestyle of the otters make them particularly susceptible to infection and disease, with proximity to fresh water rivers and runoff shown to be important risk factors for infection.(See Exhibit 1) Prey selection may also be contributing to the high otter prevalence since bivalves such as mussels have been shown to concentrate the pathogen, which could also pose health risks to other animals and humans consuming raw or undercooked shellfish.[3]

Despite dedicated recovery efforts by state and federal agencies, the threatened southern sea otter population offshore California has shown slower than predicted growth rates, growing three percent annually from 2003 to 2007 instead of the expected five percent.[4] In 2006, Governor Schwarzenegger signed AB 2485 to improve protections for the sea otter from water pollution, disease, and illegal killings. The bill specifically called for addressing threats to southern sea otters by reducing levels of the *T. gondii* parasite in wastewater released in coastal waters.

Sources of T. Gondii

The *T. gondii* parasite infects most warm-blooded animals, including humans, but the primary host is the felid (cat) family. Wild and domestic cats contract the disease, usually by eating an infected rodent, and for a number of weeks afterward, excrete the parasite through feces. The parasite is shed as an environmentally-resistant oocyst (thick-walled spore) that is extremely durable and can remain infective for over a year in soil, for several years in fresh water and for at least six months in salt water. Humans contract the parasite through various means, including hand-to-mouth contact following gardening, cleaning a cat's litter box, or contact with children's sandpits, ingesting raw or partly cooked meat that contains oocysts, or drinking water that has become contaminated with *T. gondii*.

Detection of T. Gondii

Water quality monitoring in California has traditionally focused on trace metals, pesticides and coliform bacteria as indicators of contamination; however, current monitoring efforts do not address the presence and risk of fecally-transmitted protozoal parasites such as *T. gondii* that can be difficult to detect, survive for prolonged periods of time in the environment, and cause disease in animals and humans at low doses. Currently there are standardized methods for detecting the protozoal pathogens *Giardia* and *Cryptosporidium* in water, but these methods do not detect *T. gondii*. The increasing prevalence of the waterborne pathogen and resistance of *T. gondii* oocysts to destruction by traditional methods for water disinfection (chlorine, ozone, UV irradiation) reinforces the need for improved detection and monitoring efforts.

Under the proposed project, the grantees would seek to develop and validate a water testing assay, similar to the U.S. EPA 1623 protocols for detecting *Giardia* and *Cryptosporidium*, to concentrate, isolate, and quantify *T. gondii* oocsyts in environmental water samples. If the

[2] M.A. Miller et al, 2002. "Coastal freshwater runoff is a risk factor for Toxoplasma gondii infection of southern sea otters." International Journal for Parasitology, 32:997–1006.
[3] K.D. Arkush et al., 2003. "Molecular and bioassay-based detection of Toxoplasma gondii oocyst uptake by mussels." International Journal for Parasitology, 33:1087–1097.
[4] U.S. Fish and Wildlife Service, "Final Southern Sea Otter Stock Assessment Report", December 2008. http://www.fws.gov/ventura/speciesinfo/so_sea_otter/SSO_SAR_2008_FINAL.pdf.

research and development of the assay is successful[5], this technology would allow state agencies, industry participants and non-profit organizations to use the assays for environmental testing of *T. gondii* which could be incorporated into existing water quality monitoring programs.

Project Details and Scope of Work

The project proposes to enhance existing water quality testing methods by developing monoclonal antibodies to the *T. gondii* parasite that can be used to detect the parasite within water samples using common sampling technologies. These antibodies are a natural response to an infection by the parasite, which can then be used to recognize and bind to the oocyst wall of the parasite in environmental samples. To develop these monoclonal antibodies, the grantees will immunize mice with recombinant oocyst proteins and selectively produce the antibodies. Next, the grantees will use an immunomagnetic separation (IMS) system for concentrating *T. gondii* oocsyts in water, similar to the filtration method used for recovering *Giardia* cysts. Concentrations of oocysts will then be visually detected using a direct fluorescent antibody assay (DFA), where a fluorescent molecule recognizes and binds to the antibodies attached to the parasite. Next, the sensitivity and specificity of oocyst detection will be evaluated for possible false positive reactions with bacteria and other fecal protozoa (i.e., *Giardia* and *Cryptosporidium*). Once the concentration and detection methods (IMS and DFA) are optimized, they will be tested on surface water samples collected from coastal sites identified as "high risk" for *T. gondii* exposure.

The project will be completed within two years and the research team is ready to begin immediately. During the first year, the grantees will produce and select monoclonal antibodies, and in the second year conduct the IMS and DFA optimization, water sample testing, and assay validation. Once the *T. gondii* detection methods are validated, the grantee intends to contract with a third party to commercially produce the assay and make it readily available to industry stakeholders as well as agencies such as the Department of Fish and Game and state and regional water quality boards for their use in water quality monitoring efforts. In addition, the research team is willing to conduct outreach to assist potential users with the adoption of the new *T. gondii* detection methods and incorporation into existing monitoring protocols.

Project outcomes will include annual progress reports, presentations and publications resulting from the study, and feedback from stakeholder groups on the sensitivity, specificity, and practical application of any methods developed for improved detection of *T. gondii* in water samples.

PROJECT GRANTEE:

The University of California at Davis is a major research center for animal health and has fostered extensive research into animal as well as human pathology. Specifically, the collaborative research team led by Drs. Conrad and Miller has successfully completed a variety of studies investigating the disease ecology of fecal pathogens in coastal California ecosystems. Dr. Patricia Conrad is a parasitologist whose overall research program focuses on protozoal contamination in freshwater, estuarine, and nearshore marine environments. Her research

[5] Because this is a research grant, it is assumed that a successful protocol is not guaranteed to be developed.

includes studies assessing the epidemiology of *T. gondii* in southern sea otters in California. Dr. Woutrina Miller is an epidemiologist and ecological microbiologist whose ecosystem health research has focused on both protozoal and bacterial waterborne pathogens in coastal areas. She has published on the use of bivalve shellfish as bioindicator species for protozoal contamination and the application of best management practices to reduce coastal pathogen pollution from dairy runoff.

Dr. Jim Trimmer, a professor in the U.C. Davis Department of Neurobiology, Physiology, and Behavior, will be a collaborator on this project. He has extensive experience with monoclonal antibody development using recombinant protein immunizations and will provide intellectual and technical support in the production of recombinant proteins, as well as all aspects of *in vitro* monoclonal antibody production.

SITE DESCRIPTION:

The study will provide a means of detecting harmful fecal pathogen pollution which affects aquatic ecosystems (estuarine and marine in particular) throughout California. Project outcomes will initially be applied in coastal California, in particular state waters north of Pt. Conception (southern sea otter habitat), as well as inland waterways that may be transporting the parasite from land to sea. The technology will also have application nationally and internationally as it is incorporated into existing water quality monitoring programs.

PROJECT APPLICATION:

The State Coastal Conservancy currently administers a research program, created by the Sea Otter Bill of 2006 (AB 2485) and funded through a voluntary state income tax check-off, that is carried out by the California Department of Fish and Game and U.C. Santa Cruz to study sea otter mortality from non-point source pollution. The latest study to be funded by the program is comparing two coastal sites near Monterey, one heavily impacted by human development and the other with a low level of impact, in order to evaluate rates and causes of sea otter mortality. Research includes sampling individual otters to measure pathogen, biotoxin, and contaminant exposure levels and performing necropsies and cause-of-death analyses for all otters that die during the course of the study. Using telemetry (i.e. affixing radio transmitters), the researchers are also collecting demographic, behavioral, dietary and life history data that is important to determining how the species is interacting with and impacted by changes to the coastal environment, including the presence of water-borne pathogens. The data collected will specifically be used to examine the prevalence of sea otter exposure to *Toxoplasma gondii* and identify environmental risk factors.

The proposed project would contribute to those efforts by providing the necessary water testing assay for the *T. gondii* parasite in coastal water samples. The development of the improved detection assay will thus directly benefit the ongoing sea otter research program by allowing researchers to identify the immediate areas of exposure to the fatal parasite in sea otters, and determine how these differ between an area adjacent to a landscape that has been heavily modified by people versus an area of low human impact.

Additionally, the technology produced from this project will be of value to water quality researchers and monitoring agencies in California and throughout the United States. The project

researchers have received numerous requests and letters of support from stakeholder groups interested in testing water and sewage samples for *T. gondii* in California and elsewhere (see Exhibit 2). Certain agencies, such as the state and regional water boards, may require U.S. EPA approval of the assay design before using the technology, which may delay adoption of the assay into their monitoring protocols; however, this process may be expedited as the project researchers are already working with the EPA on approving a related project. Once approved, the technology will be ready for incorporation into existing standard water quality testing methods, such as US EPA Method 1623 used to test for *Cryptosporidium* and *Giardia* in environmental water samples.

PROJECT FINANCING:

Ocean Protection Council	$380,706
Other Funding: NIH Grant to U.C. Davis	$27,523
Total Project Costs	$408,229

The anticipated source of funds for the OPC grant will be the fiscal year 2009-2010 appropriation from the Safe Drinking Water, Water Quality and Supply, Flood Control, River and Coastal Protection Bond Act of 2006 (Proposition 84). Proposition 84 authorizes the use of these funds for purposes consistent with Section 35650 of the Public Resources Code, establishing the California Ocean Protection Trust Fund (Pub. Res. Code § 75060(g)). Under Section 35650(b), Ocean Protection Trust Fund monies may be expended for projects authorized by the OPC that are identified as appropriate Trust Fund purposes. The project is consistent with the Trust Fund purposes as discussed in the following section.

This project is also appropriate for prioritization under the selection criteria set forth in Section 75060(g). Section 75060(g) provides that the Council will give priority to projects which develop scientific data needed to adaptively manage the state's marine resources. Under the proposed project, the grantees will develop new water testing methods to allow for the detection of a harmful water-borne parasite affecting the marine environment. The new methods will ultimately yield information crucial to protecting a threatened marine species, the southern sea otter, as well as other potential marine species and recreational users in coastal areas.

Additional funds will be provided by a National Institute of Health (NIH) grant administered by U.C. Davis to support a graduate student researcher to work on the study 25% of her time.

CONSISTENCY WITH CALIFORNIA OCEAN PROTECTION ACT:

This project is consistent with the California Ocean Protection Act, Division 26.5 of the Public Resources Code. Section 35615 directs the OPC to establish policies to coordinate the collection of scientific data related to the ocean and transmit the results of research and investigations to state agencies to provide information for policy decisions. The goal of the proposed project is to improve existing water quality monitoring by facilitating the detection of a deleterious water-borne pathogen, *T. gondii*, which negatively affects at least one imperiled marine species as well as humans and other animals. The new methods will allow agencies and other stakeholders to

collect scientific information regarding the location and extent of the water-borne pathogen that will ultimately contribute to enhancing the protection of the coastal marine ecosystem. The Department of Fish and Game, State Water Quality Board and several regional water quality boards, have stated that this monitoring technology is needed to detect *Toxoplasma gondii* in water samples and would be compatible with their existing sampling protocols.

The Ocean Protection Act identifies Trust Fund-allowable projects in PRC Section 35650 (b)(2) as projects that (1) improve coastal water quality, (2) address coastal water contamination from biological pathogens, and (3) provide monitoring and scientific data to improve state efforts to protect and conserve ocean resources.

The proposed project is consistent with the Trust Fund in that the study will: (1) improve water quality monitoring methods to support the state's efforts to protect and conserve ocean resources; (2) provide information about the extent of the fecal parasite, *T. gondii*, particularly in coastal areas where southern sea otters are being threatened by the pathogen; and (3) support the protection of coastal waters and ocean ecosystems through enhancing water quality monitoring methods to test for and potentially mitigate biological pathogen contamination.

CONSISTENCY WITH OPC'S STRATEGIC PLAN:

Goal C (Ocean and Coastal Water Quality), Objective 4b: Improve water quality testing programs and warning systems; Investigate options for detection and treatment of pharmaceuticals, pathogens, and endocrine disruptors in wastewater and runoff. The primary objective of the proposed project is to improve current water quality testing methods to allow the rapid detection of a fecal pathogen, *T. gondii*, which poses a significant threat to a federally listed threatened marine species, the southern sea otter, as well as humans and other animals. This approach is designed so it can be integrated with EPA-approved methods currently in use for detection of other protozoal parasites and will significantly improve the capacity of stakeholders to recognize fecal pathogen contamination in coastal waters.

Goal C (Ocean and Coastal Water Quality), Objective 2: Support the development of new technologies and approaches to reduce nonpoint source pollution. The proposed project will create an improved system of testing for the *T. gondii* parasite and thereby assist in identifying the areas and methods of transfer of the pathogen within the land-sea interface via nonpoint source pollution (agricultural and storm runoff) as well as point source pollution (sewage outfalls). The new testing technology will be transmitted to stakeholder groups, including state and regional water quality boards, to assist in current monitoring efforts to reduce pollution of the coastal environment from land-based sources.

Goal B (Research and Monitoring), Objective 1: Improve scientific understanding of our ocean and coastal ecosystems. The improved oocyst detection methods will make it possible to identify the areas most at risk for waterborne *Toxoplasma* contamination for human and wildlife populations in coastal areas, thus reducing the likelihood of infection and disease for many marine species, including the threatened southern sea otter population. Information gained through the new testing methods can help to create a baseline for measuring future changes in the extent of the pathogen within coastal ecosystems as well as identify contributing sources of fecal

pollution, and provide metrics to assess future success or failure of sea otter management measures.

CONSISTENCY WITH OPC'S GRANT PROGRAM FUNDING GUIDELINES:

The proposed project is consistent with the OPC's Grant Program Funding Guidelines adopted November 20, 2008, in the following respects:

Required Criteria

1. **Directly relate to the ocean, coast, associated estuaries, or coastal-draining watersheds:** The proposed project addresses a threat to all aquatic ecosystems and the species therein; however this study is particularly important to coastal species, such as the southern sea otter, which inhabit the land-sea interface where polluted runoff and under-treated sewage are regularly deposited.
2. **Support of the public:** See Exhibit 2.
3. **Greater-than-local interest:** The new sampling methods will provide a needed technology for investigating fecal pathogen pollution in all coastal-related areas throughout California. In addition, the methods will provide the means to obtain data that may inform science-based management and policy decisions in California and nationally. The project will also provide a critical detection and monitoring component to support the objectives of the Sea Otter Protection program, which is supported by taxpayers statewide.

Additional Criteria

4. **Innovation:** The improved oocyst detection methods will make it possible to identify the areas most at risk for waterborne *Toxoplasma* contamination of human and wildlife populations in coastal areas, thus reducing the likelihood of infection and disease for many marine species, including the threatened southern sea otter population.
5. **Improvements to management approaches or techniques:** New methods for the specific detection of *T. gondii* oocysts developed and validated in this project will allow for improved water and wastewater pathogen monitoring in coastal areas. The assay will be designed so it can be integrated with EPA-approved methods currently in use for detection of other protozoal parasites.
6. **Timeliness or Urgency:** The proposal grantees have received numerous requests from resource agencies and stakeholder groups for assistance with *T. gondii* environmental testing and recently produced adequate numbers of oocysts to begin work immediately upon specific detection methods. In addition, the faculty and staff, facilities and a doctoral student are all in place to begin work on this project; funding for the doctoral student is currently available for a limited time from a National Institute of Health grant. It is expected that the two-year timeline will provide adequate time to complete the goal of the project; in case of a failure to produce adequate antibodies for use in detecting the parasite, the researchers have prepared a back-up plan to utilize synthetic immunizations to elicit the specific immune response needed to develop the antibodies. Other than the limited NIH grant, no other funding sources are available to develop this protocol.
7. **Coordination:** Associated work already underway by this research team is funded by a National Science Foundation (NSF)/National Institutes of Health (NIH) Ecology of

Infectious Disease Grant (2005-2008) to evaluate the dynamics of *T. gondii* transmission from land to sea and a State Water Board grant (2007-2009) to improve monitoring and mitigation methods for bacteria, *Cryptosporidium* and *Giardia* pollution along the central coast of California. A major limitation of both projects is the lack of sensitive methods available to detect *Toxoplasma gondii* oocysts in water and to track their transport in the environment. The development of methods for concentrating and recovering *T. gondii* oocysts from environmental samples, as proposed in this study, will significantly advance research efforts in elucidating *T. gondii* contamination in aquatic ecosystems. This project will also provide critical information to assist the current state-funded research program underway by the California Department of Fish and Game and U.C. Santa Cruz to study sea otter mortality from non-point source pollution and identify pathogens causing sea otter mortality.

CONSISTENCY WITH OPC'S PROGRAM PRIORITIES FOR 2009 THROUGH 2010:

The proposed project fulfills the following issue area as identified in the 2009/2010 OPC Program Priorities category of Ocean and Coastal Water Quality:

Polluted Runoff- The water sample assay can be used to provide critical information on the area and extent of the water-borne fecal pathogen *T. gondii* that is transported to the coastal ecosystem via sewage outfalls and non-point source runoff.

COMPLIANCE WITH CEQA:

The proposed project is categorically exempt from review under the California Environmental Quality Act ("CEQA") pursuant to 14 Cal. Code of Regulations Section 15306 because the project involves only data collection, research and resource evaluation activities that will not result in a serious or major disturbance to an environmental resource. Staff will file a Notice of Exemption upon approval by the council.

Exhibit 1

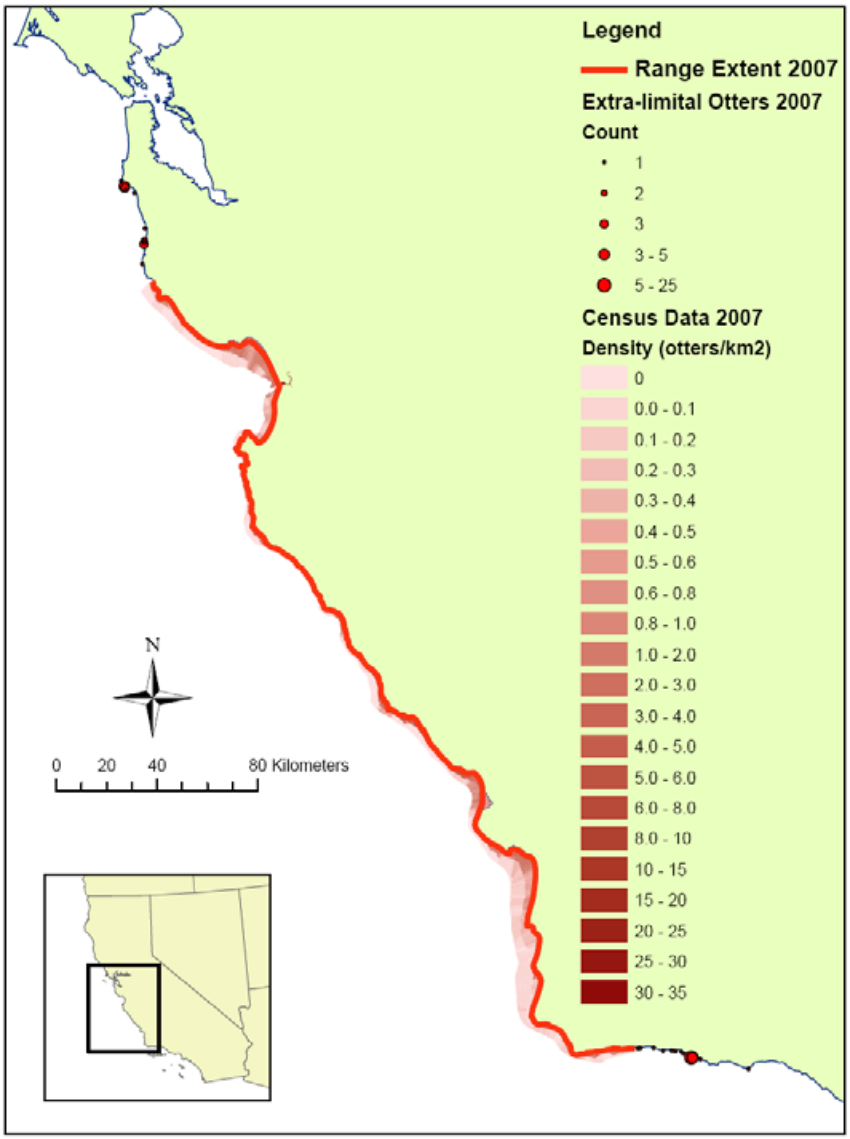

Figure 1- Current range and densities of the southern sea otter (2008 sea otter census). Data source: U.S. Geological Survey, http://www.werc.usgs.gov/otters/casurveys.html.

Exhibit 2

Tim Tinker Letter of Support to OPC (sent 10/14/08):

I am writing to express my strongest possible support for the proposal submitted to the Ocean Protection Council by Dr.s Pat Conrad, Woutrina Miller and Heather Fritz, entitled "Improved Detection of Toxoplasma gondii Oocysts." As a lead Principal Investigator in the Coastal Conservancy-supported research project "Investigating the Consequences of Coastal Contamination and Anthropogenic Stressors for Sea Otter Recovery", I am acutely aware of the need for a reliable method of concentration and detection of T. gondii oocysts in sea otters, their invertebrate prey and their environment. The proposed project is designed to accomplish exactly that goal; given the high likelihood of success in this regard, the end product will contribute substantially to the success of our own research. However, the proposed work has implications far beyond sea otter health and population recovery: it represents exactly the sort of tool that is needed to monitor human impacts on water quality and ocean health, determine how this terrestrially-derived disease is vectored into the marine environment, and ultimately provide the means for protecting the general public from a very serious health threat. I believe that the Ocean Protection Council has an opportunity to support the basic foundation that needs to be in place for effective monitoring, scientific study and mitigation of a serious threat to the health of marine species inhabiting the near-shore ocean, and to the many people that live in coastal communities.

Please do not hesitate to contact me if there is any additional information I can provide about our research and the importance of the current OPC proposal.

Sincerely,

Mr. Tim Tinker
Research Wildlife Biologist
USGS-Western Ecological Research Center
Santa Cruz Field Station

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